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Petroleum Contamination: Quantification and Passive Tagging in Organisms and Sediments

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J. W. Farrington, et al

Woods Hole Oceanographic Institution
Woods Hole, Massachusetts

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PETROLEUM CONTAMINATION: QUANTIFICATION AND PASSIVE TAGGING IN ORGANISMS AND SEDIMENTS

by

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A review of hydrocarbons in the marine environment is presented with the goal of providing a background for evaluating methods for the measurement of petroleum pollution in marine organisms and sediments. Comparisons are made of extraction, isolation and measurement procedures applied to hard shell clams (Mercenaria mercenaria) and near shore and estuarine sediments. Gas chromatographic, gas chromatographic-mass spectrometric, and U.V.-fluorescence techniques were used to analyze the hydrocarbons isolated by these procedures.

The analytical procedures were applied to surface sediments from the continental slope, shelf, and near shore area of the northeastern United States. The extent of chronic petroleum contamination was estimated as was the historical record of petroleum contamination in selected areas via depth distribution measurements in sediment cores.

The methods were also applied to intertidal marsh sediments from areas contaminated by two different No. 2 fuel oil spills. The absolute concentration changes and relative composition changes in the composition of aromatic hydrocarbons are reported for time intervals of six years after one spill and two years after the second spill. The influence of weathering processes on a few passive tagging parameters was also investigated.

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FOREWORD

The Narragansett Environmental Research Laboratory of the U. S. Environmental Protection Agency is conducting research to develop a firm, factual basis for regulatory decisions designed to protect man and his environment from damaging effects of pollutants in marine waters. Information needed in such decisions includes:

- Physical and chemical fate and transformations of pollutants in marine systems
- Responses of marine organisms to pollutant stress
- The resultant effects of different organism and community responses on the integrity of ecosystems and biogeochemical processes

This report describes the results of research directed at the first of these information needs. It presents methodology for determining the distribution of oil entering the marine environment between sediments, organisms and water; and also for relating the latter to the source of oil.

Tudor T. Davies
Director

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PREFACE

Petroleum pollution in the oceans caused growing concern in the late 1961's and early 1971's and required a variety of studies on the input, fate, and effects of petroleum in the marine environment. The need to have adequate methodology to measure one to one hundred parts per million (wet weight) of petroleum pollution in organisms and similar concentrations levels in sediments was recognized early in those studies. We undertook to research some aspects of this measurement problem. We approached the problem by first thoroughly reviewing available knowledge of naturally occurring, biosynthesized hydrocarbons and the composition of petroleum and petroleum products. We then designed experiments and evaluation tests in the laboratory and in the field for the development of methods to detect petroleum hydrocarbons at various levels in the presence of biosynthesized hydrocarbons in organisms and sediments.

We shifted priorities and the focus of the project several times during the time period of 1973-1976 when marine petroleum pollution research underwent exponential growth and immediate answers to diverse problems were demanded of us. We published our key results in a series of scientific papers and chapters and having satisfied this prime need to get the information out in a usable form, we set a relatively low priority in reworking those papers and chapters into a single coherent report - this report. Some additional delays resulted from missed communications within a hard-pressed U.S. Environmental Protection Agency which also suffered from constantly shifting priorities. One of us, John W. Farrington, accepts responsibility for the decisions causing the delay of this report and hopes that the delay did not result in extensive problems for our colleagues in oil pollution research.

Since our publication of the results of research from this project, further developments of methodology and more extensive evaluations have been undertaken by other investigators. We hope that our earlier work contributed to or at least stimulated some of the later research.

ABSTRACT

A review of hydrocarbons in the marine environment is presented with the goal of providing a background for evaluating methods for the measurement of petroleum pollution in marine organisms and sediments. Comparisons are made of extraction, isolation and measurement procedures applied to hard shell clams (*Mercenaria mercenaria*) and near shore and estuarine sediments. Gas chromatographic, gas chromatographic-mass spectrometric, and U.V.-fluorescence techniques were used to analyze the hydrocarbons isolated by these procedures.

The analytical procedures were applied to surface sediments from the continental slope, shelf, and near shore area of the northeastern United States. The extent of chronic petroleum contamination was estimated as was the historical record of petroleum contamination in selected areas via depth distribution measurements in sediment cores.

The methods were also applied to intertidal marsh sediments from areas contaminated by two different No. 2 fuel oil spills. The absolute concentration changes and relative composition changes in the composition of aromatic hydrocarbons are reported for time intervals of six years after one spill and two years after the second spill. The influence of weathering processes on a few passive tagging parameters was also investigated.

This report was submitted in fulfillment of grant No. R-802724 by Woods Hole Oceanographic Institution under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period July 1, 1973 to February 28, 1976, and work was completed as of January, 1979.

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ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

A.P.I.	--American Petroleum Institute
f_1, f_2, f_3	--fractions one, two, or three from silica gel column chromatographic fractionation of hydrocarbons
I.R.	--infrared
I.S.	--internal standard spiked into sample at start of analysis
Phyt.	--phytane
Pris.	--pristane
SCOT^R	--Support coated open tubular - refers to gas chromatographic columns. Registered trademark of Perkin Elmer Corporation
UCM	--unresolved complex mixture signal for compounds not resolved by a gas chromatography column
UV	--ultraviolet

SYMBOLS

C_{16}, C_{22} . . .	--indicates the number of carbon atoms a compound in a given class contains. The subscript indicates the number of carbon atoms. Sometimes this is indicated without "C" in some figures
^{14}C	--radioactive carbon
I_{315}	--intensity of fluorescence at 315 nanometers
ex_{290}	--fluorescence induced by excitation at 290 nanometers
^oK	--degrees Kelvin
C_1 or C_2-phenanthrene	--used to indicate the number of methyl or ethyl groups substituted on an aromatic molecule; e.g., C_2-phenanthrene is a dimethylphenanthrene or ethylphenanthrene
*	indicates automatic attenuation of off-scale peak

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We especially thank Dr. John M. Hunt of our Chemistry Department, and Dr. Paul Lefcourt and Dr. Eric Schneider of the U.S. Environmental Protection Agency, Narragansett, Rhode Island, for their confidence in and support of our research.

SECTION 1

INTRODUCTION

Petroleum and petroleum products are being used in increasing amounts, and the trend is forecast to continue for at least two decades (1). Even with the application of new technology and greater care, there will be accidental spills of petroleum and small leaks in the production, transportation, and disposal systems (1). Federal agencies such as the U.S. Environmental Protection Agency (EPA) need analytical methods for monitoring and investigating the geographical extent of such spills and leaks. Evaluation of the reliability and limitations of analytical methods is important and helps to insure valid interpretation of chemical analyses for petroleum hydrocarbons. In this project, we have dealt with some aspects of the problems of analyzing for low concentrations of petroleum in organisms and sediments (1 to 10 μg/g wet weight organism or 1 to 10 μg/g dry weight of sediment). A brief investigation was also made of the use of some passive tagging measurements to correlate the low concentrations of petroleum with possible sources.

An understanding of the biogeochemistry of hydrocarbons in the marine environment is necessary to have sufficient information about the possible sources of hydrocarbons in the samples being analyzed and to discern which are naturally occurring and which are pollutant hydrocarbons. During the period of this project, the authors participated in writing two other documents based in part on research funded by this project--a chapter in Environmental Chemistry on hydrocarbons in the marine environment (2), and a chapter in Strategies for Marine Pollution Monitoring on petroleum hydrocarbons (3). Section 4 reprints the first chapter. The contents of the second chapter are not reproduced here, however, since the document contains information from the first chapter and only brief descriptions of detailed information presented in this report.

Three other papers have described the data and conclusions from the initial experiments and observations supported by this project (4-6). This report presents a more comprehensive data set and discussion. Also reported are the results of our most recent work dealing with analyses of hydrocarbons in cores of near-shore surface sediments and a limited study of the fate of a 1974 No. 2 fuel oil spill. These last two projects were not part of the original proposal, but they were later included because they seemed to warrant more intensive investigation. The data and discussion show that this was a worthwhile reorientation of the project. Section 9 is a reprinting of a paper reporting on some of these results (Ref. 151).

In each of the sections of this report, we have tried to present a fairly complete description of the problem investigated, the methodology used, the data, and the interpretation. Though this approach results in some overlap between sections, it is not excessive and it does not detract from the contents of the report.

SECTION 2

CONCLUSIONS

1) Detection of petroleum hydrocarbons in sediments and marine organisms requires an understanding of the biochemistry and geochemistry of both naturally occurring hydrocarbons and pollutant petroleum hydrocarbons.

2) Measurement of No. 2 fuel oil hydrocarbons (and by implication, C_{15}^{+} petroleum hydrocarbons in general) in clams (*Mercenaria mercenaria*) at a concentration of 10 ppm is routinely possible if initial background concentrations of hydrocarbons are less than 1 to 2 ppm.

Precision of ± 8% to 14% relative error can be attained for concentrations of 2.0 to 10.0 µg/g wet weight.

3) Clams from estuarine areas near harbors and sewage effluents contain baseline concentrations of hydrocarbons that make measurement of 10 ppm No. 2 fuel oil spike by gas chromatography or UV-fluorescence spectrometry difficult if not impossible.

4) Comparison of Soxhlet KOH, digestion, and Vertis extraction procedures for analyses of hydrocarbons in clams gave essentially the same results.

5) Comparison of three extraction procedures for isolating hydrocarbons from sediments (methanol:benzene Soxhlet extraction with and without pretreatment with 1 N HCl, and alkaline hydrolysis extraction) yielded similar amounts of hydrocarbons.

6) A 10-ppm spike of No. 2 fuel oil in sediments from the middle region of Narragansett Bay containing 150 ppm of hydrocarbons could not be detected by gravimetric analysis. An estimated 2.9 ppm out of a possible 10 ppm No. 2 fuel oil was detected by gas chromatographic analyses.

7) The precision of analyses for hydrocarbons in sediments varied with sediment type but was less than the precision obtained for analyses of hydrocarbons in clams.

8) Gas chromatographic analyses of 10 to 100 x 10^{-9} g individual hydrocarbons was not routinely feasible with commercially obtained metal SCOT OV-1 and OV-101 columns. In particular, aromatic hydrocarbons exhibited poor chromatographic behavior.

9) Intercalibration of hydrocarbon analyses has been demonstrated as

a feasible and necessary part of multilaboratory programs for analyses of hydrocarbons in marine samples.

10) Quantitative gas chromatography mass fragmentographic analyses of aromatic hydrocarbons from subtidal and intertidal marsh sediments is routinely feasible. Detection limits are at least 0.05 x 10^{-9} g/g dry weight of sediment. Precision is 2% to 3% at the level of 50 x 10^{-9} g material injected and 12% at the 1 x 10^{-9} g level.

11) Measurement of alkanes, cycloalkanes, and phenanthrenes in a Buzzards Bay, Massachusetts, sediment core suggest that a historical record of urban air combustion products is present in these sediments. Measurements of alkanes and cycloalkanes in another core from Buzzards Bay and cores from the Hudson Channel and Gulf of Maine lend support to this hypothesis.

12) A geochemical study of the fate of No. 2 fuel oil hydrocarbons incorporated into intertidal sediments in Winsor Cove, Buzzards Bay, as a result of the Bouchard Barge No. 65 oil spill in October 1974 confirm the earlier studies of the fate of alkanes and cycloalkanes from the 1969 West Falmouth oil spill.

13) Geochemical studies of aromatic hydrocarbons in sediments from the Winsor Cove spill area show a depletion of lighter molecular weight compounds relative to the heavier molecular weight compounds over a 2.5-year period of time. Phenanthrene series compounds become more predominant. Thus the higher molecular weight aromatics should be studied in long-term effects studies.

14) Glass-capillary gas chromatography is now the method of choice for fossil fuel aromatic hydrocarbon analyses, especially when coupled with computer-interfaced mass spectrometry.

15) Passive tagging of No. 2 fuel oil incorporated into organisms and sediments at the 10 to 10,000 ppm level does not appear to be feasible based on our limited studies of gas chromatographic analyses.

16) An estimated 3.6 x 10^{-3} tons of fossil fuel hydrocarbons are dumped into New York Bight sediments each year from sewage sludge and harbor dredge spoil disposal.

SECTION 3

RECOMMENDATIONS

1) Our results are only a small portion of the experience available for analyses of hydrocarbons in marine samples. The current Outer Continental Shelf Baseline and Benchmark Studies Program of the Bureau of Land Management (U.S. Department of the Interior) involves thousands of such analyses. All of this experience together demonstrates one irrevocable fact - no one method, or even three methods, of analysis will work for all samples. Thus analytical methods cannot be set in minute detail or useless data will probably result.

The principles of analysis are well recognized and have been outlined in several sections of this report. Certain methods have been shown to yield useful results for selected types of samples, and these should be used when applicable.

2) Glass-capillary gas chromatography offers high resolution, precision, and reasonable detection limits. It is the method of choice for hydrocarbon analyses, as it offers much more information content than other gas chromatography, infrared, or UV-fluorescence measurements. The latter two may be used for some routine monitoring, but only when supplemented by glass-capillary gas chromatography.

3) Intercalibration of hydrocarbon analyses among laboratories, especially government laboratories involved in monitoring surveillance, is urgently needed.

4) Low-level fossil fuel contamination of some coastal areas has clearly occurred over the past several decades, but little is known of the long-term effects. Sediments are known to contain fossil fuel hydrocarbon pollutants, but it is not known how or if they are released to the overlying waters or taken up by benthic organisms. More research is needed in this area.

5) The existence of urban air fossil fuel aromatic compounds in coastal sediments invokes such questions as: How did they get there? What are the relationships between their concentration in the environment and fossil fuel combustion processes? These are areas of research that should be pursued immediately, as change to more coal combustion might increase the flux of these compounds to the coastal environment and have adverse effects.

6) Stronger emphasis is needed on studies of biogeochemical processes in estuarine and coastal areas. Most of the significant findings of the

research supported by this project evolved from our coupling of analytical methodology with geochemical research and applying geochemical knowledge and interpretation to the data.

SECTION 4

HYDROCARBONS IN THE MARINE ENVIRONMENT*

1. Introduction

The importance of the study of hydrocarbons found in the marine environment has been set forth by several authors (7-11). Hydrocarbons in sediments have received considerable attention because of the importance of understanding the origin of crude oil. Research concerned with hydrocarbons in the marine environment has intensified during the past few years because of a substantial commitment towards understanding oil pollution problems. Published scientific literature dealing with this topic has rapidly expanded and has been accompanied by a proliferation of other publications such as technical reports and government documents. This report focuses on the literature of the period 1971-1973 and refers to other reviews where possible.

This report is concerned primarily with the hydrocarbons with 11 or more carbon atoms. We have not reviewed the literature dealing with low molecular weight hydrocarbons, i.e. methane to decane. Analytical techniques and the relative importance of processes governing the input and distribution of low molecular weight hydrocarbons compared with higher molecular weight hydrocarbons are sufficiently different to warrant the separate treatment we have set forth here for the higher molecular weight hydrocarbons. Much of our discussion concentrates on the methods of obtaining the data because an understanding of the distribution and fate of hydrocarbons in the marine environment is very much dependent on the validity and usefulness of these methods.

While omissions are inevitable, we think we have discussed the major advances in research dealing with this topic.

2. Origin of Hydrocarbons

Hydrocarbons enter the marine environment via three general processes.

Biosynthesis - Marine and land organisms biosynthesize hydrocarbons either *de novo* or from ingested precursor compounds. These hydrocarbons may be released during metabolism or upon the death and decomposition of the organisms. Estimates of the rate of biosynthesis of hydrocarbons by

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marine primary productivity are generally given as 1-10 million metric tons per year (12, 13). Estimates of the total input to the marine environment by land organisms are not available. However, substantial quantities of hydrocarbons are released from land plants to the atmosphere (14, 15) where they are subject to deposition on land and on sea, and to photochemical and chemical alteration. The actual amount reaching the world's oceans is unknown.

Geochemical Processes - Submarine and coastal land oil-seeps release petroleum hydrocarbons to the marine environment. The annual input rate is variously estimated at between less than 0.1 million metric tons (19) and 10 million metric tons (17). A recent review of this subject has arrived at an annual input rate of 0.6 million metric tons per year (18).

Weathering of soils and sediments and transport of some of the hydrocarbons in these sediments to the marine environment should also be considered as an input, although probably small relative to other sources because of slow degradation of the hydrocarbons during the weathering process. No estimates of the annual rate of input are available.

There are chemical synthesis processes which are sources of hydrocarbons. Forest fires inject an estimated 6 million metric tons of hydrocarbons per year (13) into the atmosphere. An unknown portion is eventually delivered to the marine environment. There are also chemical reactions occurring during the diagenesis of organic matter in sediments which yield hydrocarbons. These hydrocarbons may eventually find their way into the present marine environment either by submarine exposure of sediments or by diffusion out of the sediments. No estimates are available as to rates of input, however, the annual rate is probably small.

Anthropogenic Inputs - The inputs of hydrocarbons from accidental and intentional discharge of fossil fuel to the marine environment and the input of hydrocarbons from the atmosphere have been estimated and recently reviewed in detail (18, 19). The annual input rate is of the order of 1-10 million metric tons. The uncertainties of several of the estimates should be emphasized as well as the fact that these are global averages and may be quite different for any given location and any given point in time.

3. Analysis of Petroleum Hydrocarbons and Biosynthesized Hydrocarbons

One of the critical problems for analysts of hydrocarbons in marine samples is the detection of petroleum hydrocarbons in the presence of recently biosynthesized or native hydrocarbons. The methods of analysis for bulk oil samples and slicks resulting from accidental spills have been discussed and reviewed by other authors (20). Techniques for analysis of recently biosynthesized hydrocarbons in marine organisms from the viewpoint of elucidation of chemical structure have been reviewed (9, 20).

Comparison of the Composition of Petroleum Hydrocarbons and Biosynthesized Hydrocarbons - The detection of petroleum hydrocarbons in many marine samples is complicated for two principal reasons. First, the analyst must distinguish between recently biosynthesized or native hydrocarbons and petroleum hydrocarbons in order to estimate petroleum contamination. The distinction can only be made by comparing hydrocarbons native to marine organisms with petroleum hydrocarbons to discern the characteristics of each group. Secondly, hydrocarbons in petroleum give such a wide range of molecular structures and molecular weights that no presently available method of analysis provides an accurate assessment of total petroleum contamination when considering all possible oil-contamination incidents.

Petroleum Hydrocarbons (see Figure 1). There are several classes of hydrocarbons in crude oils and most crude oil products. The distribution of hydrocarbons in these classes is briefly presented below. In general, the discussion of this subject by Blumer et al. (20) has been followed.

n-Alkanes. A series of n-alkanes extending from C_1 beyond C_{60} is present, with a ratio of abundance of odd to even-carbon-numbered chains of ca. 1.0. However, dewaxing removes most n-alkanes up to about n-C_{40} from lubricating oils.

Branched Alkanes. Many parallel homologous series of isomers, including parallel homologous series of isoprenoid alkanes, are present in petroleum.

Cycloalkanes (naphthenes). A complex mixture of compounds including substituted and unsubstituted rings is present, with the substituted compounds more abundant than their parent compounds.

Aromatic Hydrocarbons. Again a complex mixture of compounds is present, including mono- and poly-alkylbenzenes, naphthalenes, and polynuclear aromatic hydrocarbons with multiple alkyl substitution. Also included in this class are compounds sometimes designated as naphtheno-aromatics owing to the mixed nature (aromatic, cycloalkane) of the subunits.

Alkenes (olefins). These compounds can be present in refinery products but are generally absent in crude oils (20).

Several authors have discussed the hydrocarbon composition of crude oils and crude oil products in detail (21).

Recently Biosynthesized or Native Hydrocarbons (see Figure 2). Some hydrocarbons in marine organisms may come from the ocean, either picked up from the water directly through permeable surfaces such as gills or obtained along with food through absorption from the gut (22, 23). Hydrocarbons are more stable than most biochemicals, so it would not be unexpected to find some derived from land sources in marine organisms. Organisms can also make their own hydrocarbons, either de novo or from precursor compounds obtained with their food. An example of the latter is the conversion of phytol into pristane (18). Non-pollutant hydrocarbons from these sources are all of recent biosynthetic origin; they may all be called native to organisms.

The most recent comprehensive review of hydrocarbons native to organisms was that of Gerarde and Gerarde in 1961-1962 (25). In 1966, Clark (26) re-

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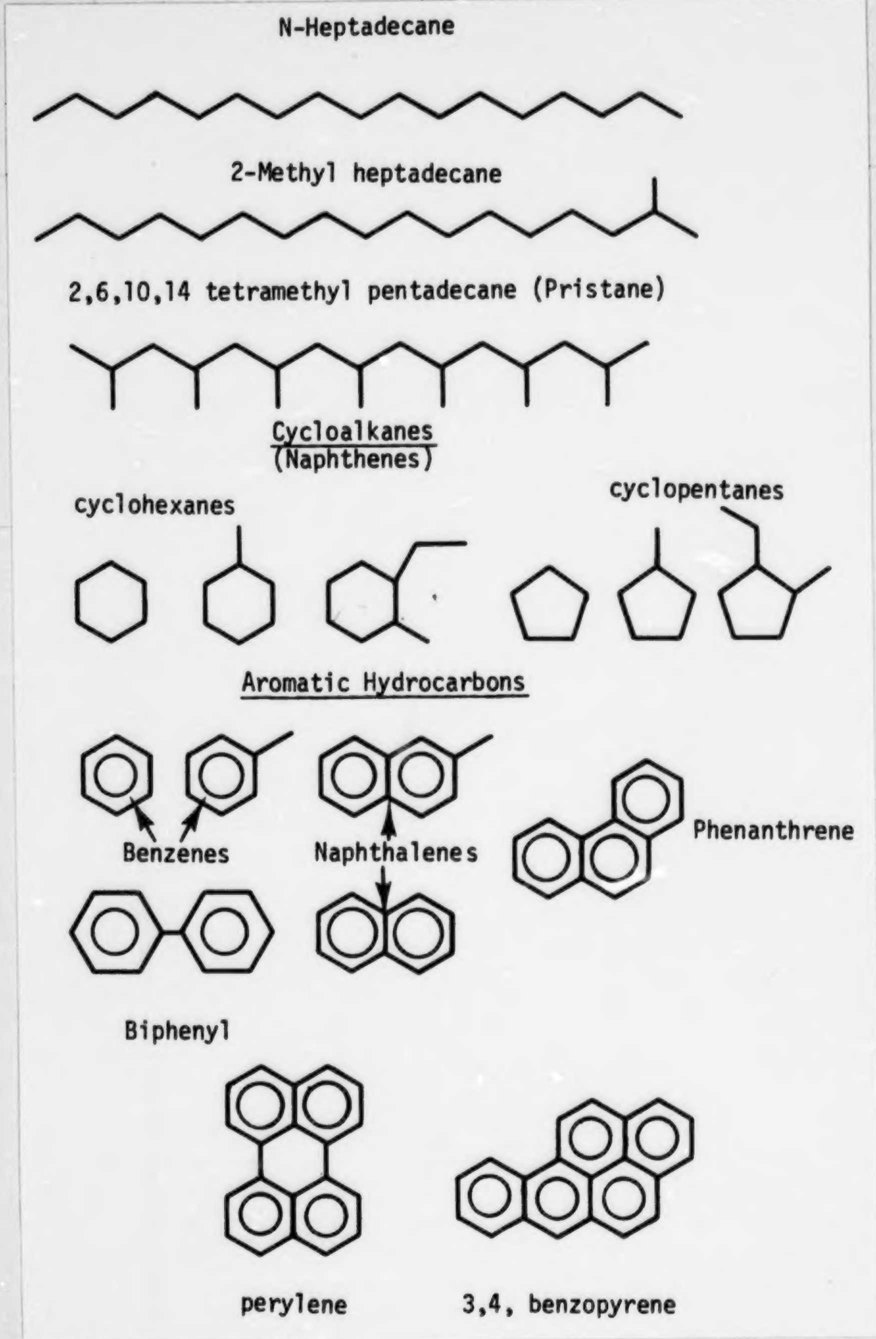
N-Heptadecane
2-Methyl heptadecane
2,6,10,14 tetramethyl pentadecane (Pristane)
Cycloalkanes
(Naphthenes)
cyclohexanes
cyclopentanes
Aromatic Hydrocarbons
Benzenes
Naphthalenes
Phenanthrene
Biphenyl
perylene
3,4, benzopyrene

Figure 1. Structure of some petroleum hydrocarbons

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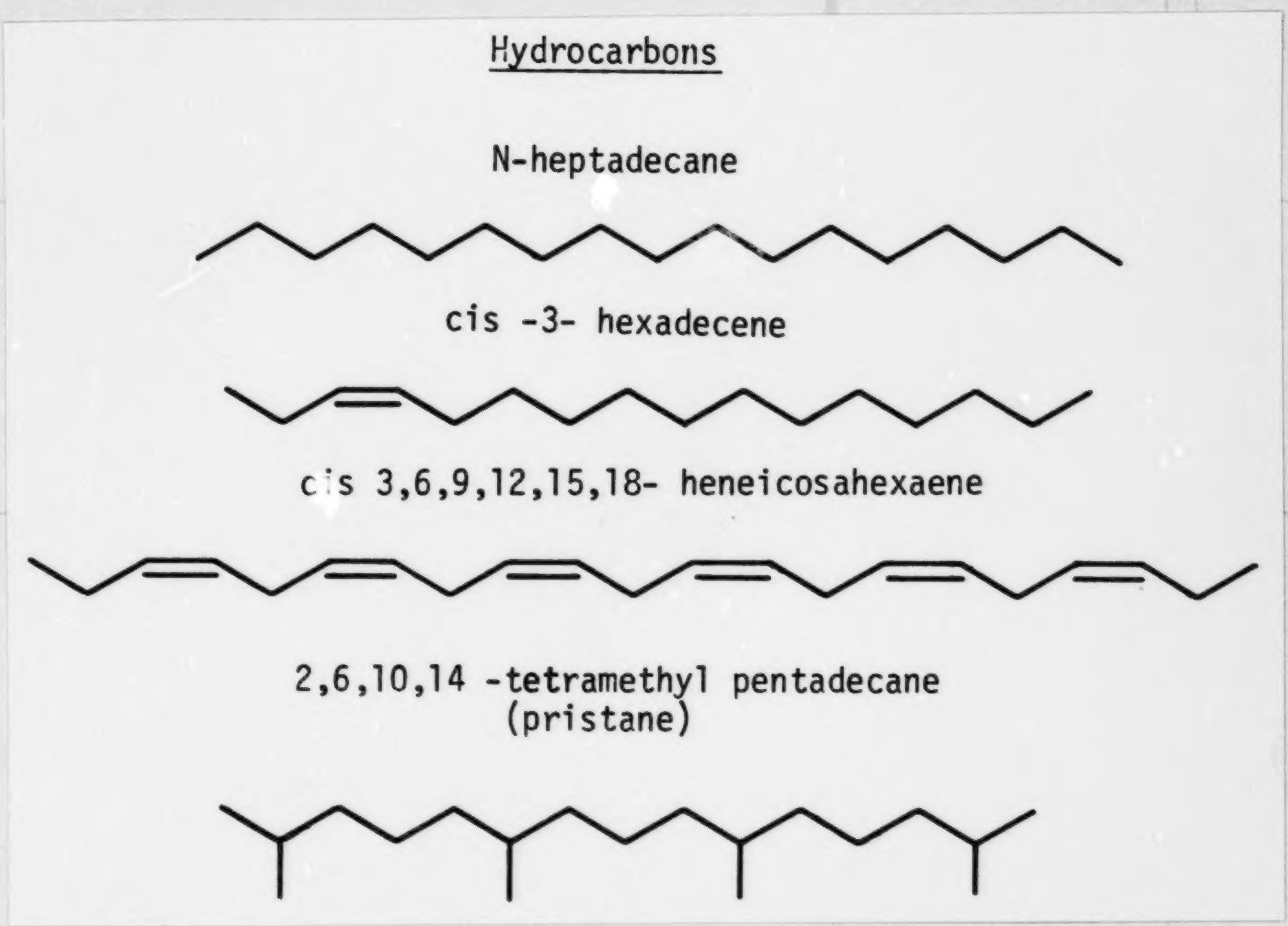
Hydrocarbons
N-heptadecane
cis -3- hexadecene
cis 3,6,9,12,15,18- heneicosahexaene
2,6,10,14 -tetramethyl pentadecane
(pristane)

Figure 2. Structures of some biosynthesized hydrocarbons.

viewed the literature on saturated hydrocarbons (mostly non-cyclics).

n-Alkanes. Both land and marine organisms synthesize n-alkanes. Odd-carbon-number chains predominate, although even-carbon-number chains are present. In many instances one or two odd-carbon-chain n-alkanes predominate over all others. In marine phytoplankton, the n-alkanes with 15, 17, 19 and 21 carbon atoms are most abundant (26), whereas in marsh grasses and Sargassum 21-29-carbon-atom n-alkanes predominate (26, 27). Some bacteria have been found to contain equal amounts of even-carbon-number and odd-carbon-number n-alkanes between n-C_{25} and n-C_{32} (28). Marine sponges and corals have also been shown to have little if any odd-carbon preferences for the C_{25}-C_{34} n-alkanes (29).

Branched Alkanes. Several branched alkanes have been found in organisms. Pristane is the most abundant alkane in some fishes (23). Phytane has not been detected in copepods from the Gulf of Maine nor in basking shark liver (23). However, phytane has been reported to be present in bacteria (30, 31). A biochemical pathway for converting phytol into pristane but not into phytane has been demonstrated (24). Several monomethyl branched alkanes (31, 32) have been found in organisms and an alkylcyclopropane has been tentatively identified (30, 32).

Alkenes (olefins). Alkenes are often a major portion of the hydrocarbons found in organisms, particularly marine organisms. Squalene is the major hydrocarbon constituent of basking shark liver oil and cod liver oil. Isoprenoid C_{19} and C_{20} mono-, di-, and tri-olefins are present in copepods and some fishes (33). Several straight-chain mono- to hexa-olefins have been found in marine organisms (32-35). Carotenes are polyolefins and are found in considerable quantities in many organisms.

Cycloalkanes and Cycloalkenes. Hydrocarbons containing one to three non-aromatic rings are present in several herbs and other terrestrial plants (25). Most are classified as terpenoids because of their structural relationship to isoprene.

Aromatic Hydrocarbons. There are several reports of aromatic hydrocarbons having been isolated from terrestrial plants and species (25). ZoBell (36) has recently reviewed reports regarding the occurrences of polynuclear aromatic hydrocarbons in the marine environment. Some of the work cited suggests that some polynuclear aromatic hydrocarbons may be synthesized by marine microorganisms (36). The aromatic hydrocarbons native to organisms contain at most one or two substituents (35). Spectrophotometric and mass spectrometric analysis of hydrocarbons isolated from the marine mussel Mytilus edulis from one location did not detect any aromatic hydrocarbons (32).

Summary. Analyses of marine and land organisms have shown that many different types of hydrocarbon are produced by organisms. However, an analysis of an individual species does not reveal more than a few members of each homologous series. Many times only one class of hydrocarbon and in a few cases only one individual hydrocarbon predominates. The only exceptions to this generalization might be the microorganisms other than phytoplankton, i.e. bacteria, yeasts, actinomycetes. Some of the reports of the analysis of hydrocarbons in bacteria (10, 30, 32) contain gas chromatograms which suggest that the saturated hydrocarbons have a wider range of molecular weight and structure than has been found in other organisms. There is a need for further analysis of these microorganisms cultured under conditions which would preclude contamination by petroleum hydrocarbons.

It is important to note that only a limited number of terrestrial and marine species from only a few geographical locations have been analyzed for their native hydrocarbons. The stage in the life cycle of the organism at the time of analysis, the culturing conditions, and the health of the organism should also be considered since such factors can influence the hydrocarbon distribution (27, 30, 34).

Many investigators have limited their analytical techniques in order to search for only one or two classes of hydrocarbon - usually alkanes and alkenes - and would not have detected other classes, if present, or have not reported their presence. This is particularly true with respect to aromatic hydrocarbons and to a lesser extent cyclic alkanes.

Characteristics of Petroleum Hydrocarbons Useful for Detecting Petroleum Contamination. A comparison of the hydrocarbons in petroleum with hydrocarbons native to organisms shows the following differences which are useful in detecting petroleum contamination. Some of these differences may not apply for all organisms or all fossil fuels.

(i) Petroleum contains a much more complex mixture of hydrocarbons with a much greater range of molecular structures and molecular weights than has been reported for hydrocarbons native to organisms.

(ii) Petroleum contains many homologous series. Adjacent members of a series usually are present in nearly the same concentration The unity ratio of even- and odd-numbered n-alkanes is an example.

(iii) Petroleum contains numerous cycloalkanes and numerous aromatic hydrocarbons in comparison with the small number of each class which are native to marine organisms. In particular the numerous alkyl-substituted ring compounds have not been reported in organisms. Examples are the series of mono- and poly-alkylated benzenes and naphthalenes.

(iv) Petroleum contains numerous naphtheno-aromatic hydrocarbons. These compounds have not been found in organisms.

In addition to the differences in hydrocarbon composition noted above, petroleum contains hetero-compounds (S, N, O, or metals) and heavy asphaltic compounds. The numerous hetero-compounds have not been found in organisms, and heavy asphaltic compounds are not native to organisms.

There are situations where it is much more difficult to distinguish between petroleum hydrocarbons introduced by man and the hydrocarbons from natural sources. The analysis of water organisms, or sediments taken from areas near natural oil seeps is one of the more difficult situations. It is possible that extensive chemical analysis would allow an estimate of the contributions from these two sources. In some cases it appears that it may be possible to identify sources for individual hydrocarbons. Recent analyses of the stereochemical isomers of pristane from two different sources suggest that this type of analysis may provide a clue as to the relative contributions from different sources (9, 37).

If ca. 0.5 g or more of hydrocarbons can be isolated from a sample, they can be dated using ^{14}C dating techniques. An age much older than Recent (10,000 years) indicates a predominance of petroleum hydrocarbons. This assumes that recently biosynthesized hydrocarbons did not have a fossil carbon source and that the contribution of hydrocarbons from weathering of ancient sediment is negligible. This technique has been used in the analysis of hydrocarbons isolated from surface sediments in a harbor area (38).

Sampling and Analysis

Sample Contamination. Samples taken in areas not immediately adjacent to shores must be obtained from a sampling platform, usually a ship. A ship is a floating localized source of hydrocarbons. Machinery is lubricated, bilges contain oily wastes which are periodically discharged, fumes are discharged from the exhaust funnel, and winch wires on which sampling gear is suspended are usually lubricated. Constant vigilance and special precautions are needed to obtain a sample not contaminated by the ship's environment. The same vigilance and precautions are needed to avoid contamination if samples are analyzed on board the ship. Controls involving all aspects of the extraction and analytical procedure should be processed to be certain that contamination has been avoided. Periodic samples of the ship's fuel, bilges, and lubricating oils should be obtained. Comparison of hydrocarbon composition of these potential contaminants with the hydrocarbons isolated from the samples will provide a means of determining if contamination has occurred (39).

Intercalibration and Comparison of Data. Hydrocarbons in marine samples have been analyzed by a number of laboratories employing several different extraction procedures. Comparison and integration of the results of these analyses to obtain a coherent view of hydrocarbon distribution in the marine environment assumes that the inter-laboratory accuracy and precision allows such a comparison and integration. A first attempt at the intercalibration of hydrocarbon analyses of marine samples has been reported (40). Further and more extensive efforts are needed.

Extraction. Extraction of hydrocarbons from all marine samples has involved the same basic procedure. Organic solvents are used to extract hydrocarbons. The extracts also contain all or a portion of the lipid material present in the sample and require the further isolation of the hydrocarbons from the other lipid material.

Several extraction methods have been used to isolate hydrocarbons from organisms. Extraction with organic solvents using a Soxhlet apparatus, or using a homogenization procedure, has been described (e.g. refs. 40 and 41).

Connel (40) extracted samples of fish with diethyl ether followed by steam distillation of the ether extract to obtain a volatile fraction, which in the particular case cited was primarily kerosene. Some analysts advocate digestion of the sample in alcoholic KOH followed by partitioning of the hydrocarbons and unsaponifiable lipids into a non-polar solvent such as hexane (43, 44). The advantages of the digestion procedure are said to be disruption of cells and more efficient extraction of the hydrocarbons from the sample. Steam distillation has also been used to extract hydrocarbons from fish (45).

Particulate matter isolated from seawater would probably be extracted using many of the procedures reported above. The isolation of the particulate matter from seawater presents some problems. The filters employed should be thoroughly checked to be certain that they are not adsorbing hydrocarbons from the seawater in addition to removing the particulate matter.

Solvents such as CH_2-Cl_2, CCl_4, n-C_6H_{13}, n-C_5H_{12}, Freon 113, and stabilized $CHCl_3$ have been used to extract seawater samples, including surface slicks (8, 38, 46-55). It has been suggested that procedures employing an XAD-2 resin to isolate polychlorinated biphenyls from seawater might also be used with some modifications to isolate hydrocarbons from seawater (56). Polyurethane foam plugs which have been used successfully for isolating chlorinated hydrocarbons from seawater might also be used effectively for isolating hydrocarbons (57).

Some of the procedures used for extraction of organisms are also useful for the extraction of sediment samples. Organic solvent extractions, usually aided by Soxhlet apparatus, sonication, or ball-milling, have been employed (7, 37, 40, 58, 60). Elemental sulphur is present in most sediments and is co-extracted with the hydrocarbons. The sulphur is usually removed from the extract by reaction with copper (39, 61).

Extensive comparisons of the efficiency and selectivity of the various extraction procedures for marine samples have not been conducted and are needed.

Saponification. Most extracts, especially extracts of organisms obtained with the above methods, contain esters of fatty acids, e.g. waxes and glycerides. The esters often interfere with the isolation of alkenes and aromatic hydrocarbons. Saponification breaks the esters into fatty acid salts and alcohols which are easily removed, but transesterification of the existing esters to methyl or ethyl esters can sometimes occur (62). This must be avoided to prevent interference. Complete saponification is confirmed by the absence of the absorption band for esters in infrared spectra of the material remaining after saponification.

Separation of Hydrocarbons from Other Lipids. A variety of techniques have been used to separate hydrocarbons from the lipids co-extracted by the procedures discussed above. Thin-layer chromatography and column chromatography, singly and in combination, have been routinely used effectively to isolate hydrocarbon fractions from extracts (37, 44, 58-60, 63-69).

High-pressure liquid chromatography is a useful technique which should gain wider acceptance after further research and testing. Hites and Biemann (50) have successfully used it to separate aromatic hydrocarbons according to ring number. Karger et al. (70) have used 2,4,7-trinitrofluorenone-impregnated Corasil I high-pressure liquid chromatography columns to separate isomers of polynuclear aromatic hydrocarbons.

Gel permeation chromatography has been suggested as a technique which would be useful to the analysis of high molecular weight components of petroleum in marine samples (20).

Analysis of Hydrocarbons. We shall discuss each technique as to its merits for detecting petroleum hydrocarbons in the presence of comparable or higher levels of native hydrocarbons.

Infrared Spectrometry. The absorption frequencies of native hydrocarbons overlap or coincide with the absorption frequencies of petroleum hydrocarbons, with the possible exception of absorption frequencies for aromatic hydrocarbons of petroleum in the long-wavelength region. Thus, this technique shows little promise for detecting small quantities of petroleum hydrocarbons in the presence of hydrocarbons native to organisms (71).

UV Absorption and UV Fluorescence Spectrometry. These analytical techniques detect the presence of aromatic hydrocarbons. The use of UV absorption and UV fluorescence techniques as an indicator of oil contamination has been described (46, 52, 72). They can be applied to lipid extracts and unsaponifiable lipid extracts without isolating the hydrocarbons from the lipids (46, 52, 72). Appropriate attention must be directed towards the possible presence of native aromatic hydrocarbons and the quenching of the fluorescence emission.

The limitations of these techniques are as follows. There is little indication of the complexity of molecular weight range of the mixture. There is the possible overlap of the UV absorption spectra of highly conjugated native alkenes with the absorption spectra of the aromatic hydrocarbons. This limitation may be overcome by selectively hydrogenating the alkenes. Finally, no indication of the presence of alkanes or non-conjugated alkenes is given.

Gas Chromatography (see Figure 3). This technique has been useful in analyzing samples for petroleum contamination (38, 39-43, 45, 47, 50, 58-60, 63-68, 43, 74). Hydrocarbons are tentatively identified by co-injection of authentic standards or by determining Kovats retention indices of peaks in the gas chromatograms (35). The presence of an unresolved complex mixture of hydrocarbons in medium- and high-resolution gas chromatograms has been used as an indicator of the presence of petroleum hydrocarbons. This unresolved complex mixture of hydrocarbons with a wide molecular weight range has not been detected in organisms free of petroleum hydrocarbons, with the possible exception of bacteria as previously discussed.

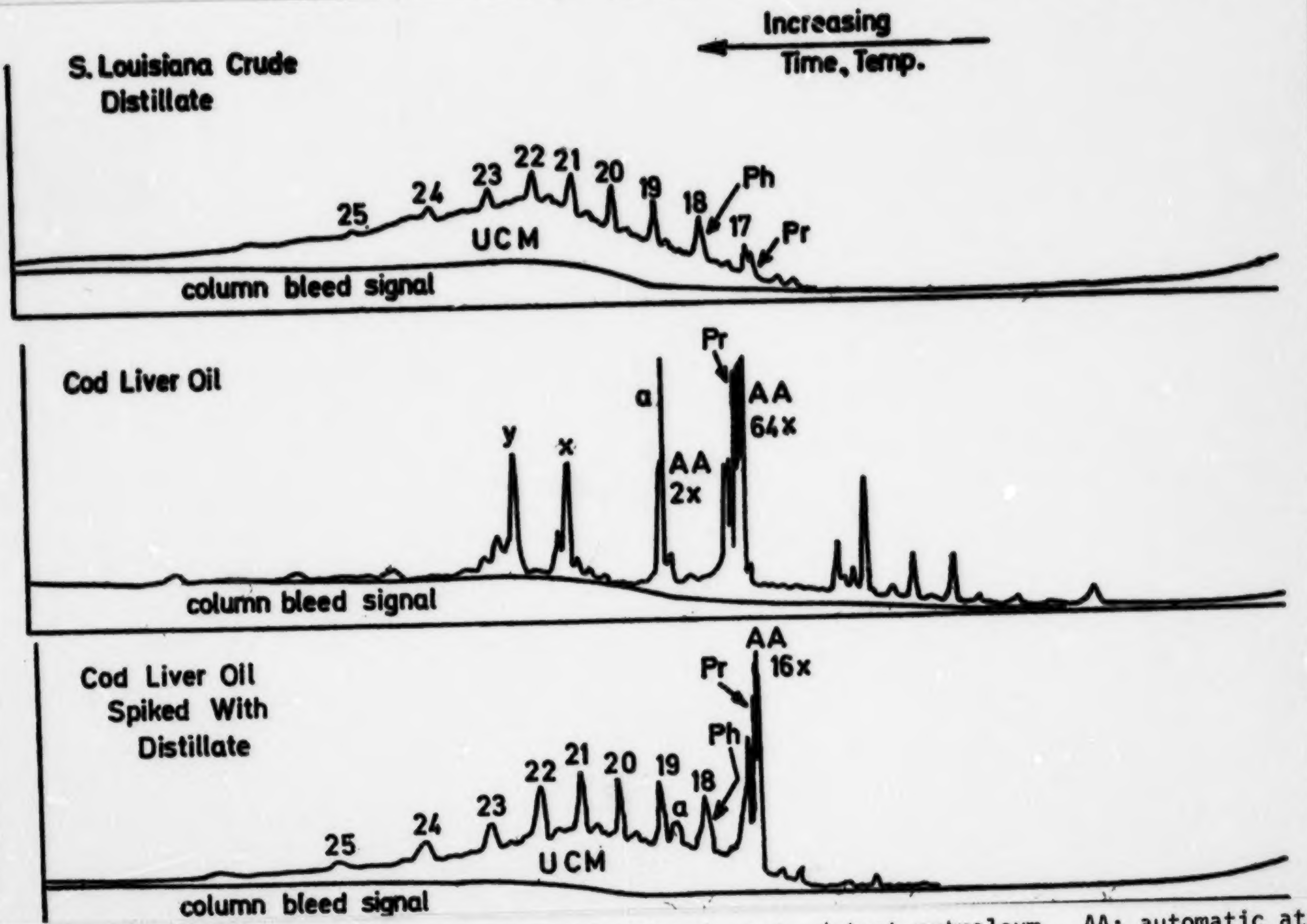

Figure 3. Gas chromatographic analysis of hydrocarbons to detect petroleum. AA: automatic attenuation of detector signal. Pr: pristane; Ph: phytane; numbers: n-alkanes; a: phytadienes; x,y: unknown hydrocarbons; UCM: unresolved complex mixture. Conditions: 3% Apiezon L, 2.3 m on chromasorb W 80/100 mesh; carrier gas N_2 at 10-15 ml min^{-1}; column was temperature programmed from 80 to 290^0 at 6^{0}C min^{-1}.

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In several cases further analysis by GC-MS was employed to confirm identifications.

Mass Spectrometry and Gas Chromatography-Mass Spectrometry. Hydrocarbons isolated from marine samples contaminated by petroleum have been analyzed by GC-MS and by mass spectrometry alone (38, 47, 50, 54, 63, 66, 73, 74). This is particularly effective for detecting petroleum contamination when low-voltage mass spectrometry is used to analyze the aromatic fractions isolated from a sample. The mass spectra obtained show nominal mass numbers of fragments of aromatic hydrocarbons, e.g. in the case of aromatic hydrocarbons from a sample contaminated with petroleum, fragments of the series of alkylated aromatic hydrocarbons of a distribution and complexity similar to that found in petroleum itself are present in the mass spectra.

Computer-Interfaced Mass Spectrometry and Gas Chromatography-Mass Spectrometry. The data-manipulating ability of a computer interfaced with a mass spectrometer or a GC-MS system creates a potentially powerful analysis system for identifying petroleum contaminants in the marine environment. The mass spectral data might be analyzed by compound-type analysis (75, 76), although validation of these techniques for the analysis of petroleum hydrocarbons in the presence of equal or greater quantities of native hydrocarbons has not been documented. Recent applications of these techniques for the analysis of hydrocarbons isolated by column chromatography from marine sample extracts have been described (47, 50, 54, 73).

Quantification. The measurement of the concentrations of hydrocarbons in the various types of sample has been accomplished by a variety of techniques. Spectrometric quantitative analyses are made using calibration curves of a representative crude oil or bunker C oil concentration in an appropriate solvent plotted versus intensity of absorption or emission (46, 54). Gas chromatographic analyses are quantified using internal standards or by calibrating the detector response using a hydrocarbon mixture of known concentration (39, 40, 43). Quantities of hydrocarbons in column or thin-layer chromatography fractions have been determined using analytical balances, including the Cahn electrobalance for very small quantities of the order of 10-500 μg (30, 38-40, 64, 67). Spectrometric measurements have also been employed to quantify hydrocarbons in fractions isolated by column chromatography (54).

Application of the Methods of Analysis. Figure 4 presents an outline of analytical techniques presently available which can be used to detect and estimate petroleum hydrocarbon contamination of marine samples. The development of new methods and new applications of existing methods can be expected to proceed rapidly as more analysts tackle the problems involved in analyzing marine samples for petroleum contaminants.

These techniques provide information in each of the categories of characteristics for petroleum hydrocarbons described earlier. Gas chromatography provides information about the complexity and the molecular weight range. It also detects the presence or absence of certain homolo-

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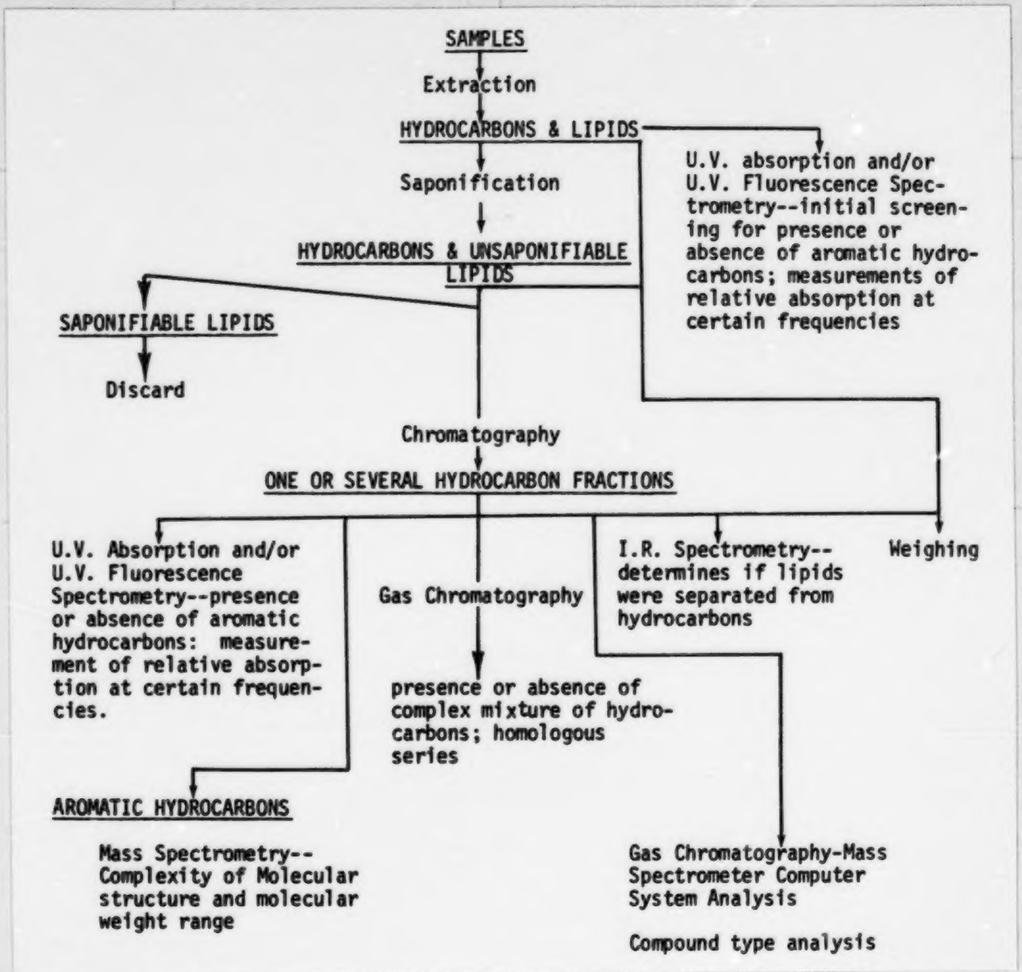
SAMPLES
Extraction
HYDROCARBONS & LIPIDS
U.V. absorption and/or U.V. Fluorescence Spectrometry--initial screening for presence or absence of aromatic hydrocarbons; measurements of relative absorption at certain frequencies
Saponification
HYDROCARBONS & UNSAPONIFIABLE LIPIDS
SAPONIFIABLE LIPIDS
Discard
Chromatography
ONE OR SEVERAL HYDROCARBON FRACTIONS
U.V. Absorption and/or U.V. Fluorescence Spectrometry--presence or absence of aromatic hydrocarbons: measurement of relative absorption at certain frequencies.
Gas Chromatography
I.R. Spectrometry--determines if lipids were separated from hydrocarbons
Weighing
presence or absence of complex mixture of hydrocarbons; homologous series
AROMATIC HYDROCARBONS
Mass Spectrometry--Complexity of Molecular structure and molecular weight range
Gas Chromatography-Mass Spectrometer Computer System Analysis
Compound type analysis

Figure 4. Outline of methods of analysis for hydrocarbons in marine samples.

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gous series, e.g. 1:1 ratio of even- and odd-carbon n-alkanes. In addition, gas chromatography provides some information about native hydrocarbons and their absolute and relative abundances.

The n-alkanes from petroleum may be hard to detect in the presence of native n-alkanes such as are found in bacteria, marsh plants, sponges, and corals. The absence of n-alkanes may mean that the sample does not contain petroleum contamination. This can be confirmed by the absence of other characteristics for petroleum hydrocarbons, or it could mean that the petroleum contaminants have been bacterially altered prior to or after incorporation into the organism. n-Alkanes are much more susceptible to bacterial attack than are other petroleum hydrocarbons (see later section).

The techniques also provide information about the presence or absence of aromatic hydrocarbons and their structural complexity and molecular weight range. Thus, we believe that careful application of these techniques will provide information about the presence or absence of petroleum contamination in marine samples and also provide an estimate of the severity of contamination.

Reporting Results of Analyses. The following information should be included when reporting results of analyses of marine organisms for petroleum contamination: (i) precision and accuracy of the method employed; (ii) limits of detection at the precision and accuracy stated; (iii) molecular weight range and molecular structure range over which the methods are precise and accurate: and (iv) molecular weight range and molecular structure range of the reported petroleum contamination in the sample.

4. Distribution of Hydrocarbons

Marine Organisms - We have reviewed most of the literature concerned with hydrocarbons found in marine organisms in an earlier section (7, 8, 10, 20, 22-40). Little research has been focused towards the understanding of the biological role of hydrocarbons in marine organisms. However, the role of squalene as an intermediate in the biosynthesis of steroids is described in the biochemical literature, as is the role of the carotene pigments. It has also been suggested that squalene, which is present in high percentages in the livers of deep-sea sharks, assists the sharks in achieving buoyancy (77). This has also been suggested as a biological role for pristane in certain species of zooplankton (78). The higher molecular weight C_{25}, C_{27}, C_{29}, and C_{31} carbon n-alkanes found in the marsh grasses probably act as a waxy outer protective layer.

The work of Blumer and co-workers and others indicates that hydrocarbons can be used as a chemotaxonomic tool and as a tool to elucidate food chains (23, 24, 26-35, 78-84). In one study (79) evidence was presented that hydrocarbons can be used as markers to distinguish subpopulations of fish which feed in different locations and therefore may ingest different assemblages of hydrocarbons. Several of the same workers have pointed to the restraints placed on the chemotaxonomic value of hydrocarbons by the variation of hydrocarbon composition with the growth stage of organisms (26, 33).

The pathways for biosynthesis of hydrocarbons in marine organisms deserve further attention. It has been established that pristane in certain zooplankton species (calanoid copepods) is derived from the phytol moiety of chlorophyll of the ingested phytoplankton (24). The correlation of the structure of the C_{22} fatty acid docosahexaenic acid and the C_{21} hydrocarbon heneicosahexaene in certain phytoplankton species suggests an inter-relationship between fatty acid biosynthesis and hydrocarbon biosynthesis (34). However, heneicosahexaene is present in benthic algae, and attempts to find the C_{22} fatty acid in three species of brown benthic algae have failed to detect the fatty acid (35). This suggests that no close inter-relationship between fatty acid biosynthesis and hydrocarbon biosynthesis exists in some cases.

It has been observed that the percentage of males produced in laboratory-reared Calanus helgolandicus, a marine zooplankton, depends on the algal composition of their food (83). Youngblood et al. (30) commented on the intriguing correlation between the percentage of males produced and the predominance of heneicosahexaene in the algal food. These workers later demonstrated (35) that the alkene:alkane ratio is linked to the stage of tissue development in the benthic alga Ascophyllum nodosum (35). Their comment . . ."we should no longer consider all plant hydrocarbons as accidental by-products of the cell biochemistry" offers a challenge to further research.

Seawater analyses - A wide variety of analytical techniques has been utilized for analyses of seawater (8, 46-52, 54-55). This makes it difficult to compare results. A large proportion of the data available has been obtained using UV-fluorescent analysis of lipid extracts of seawater (46, 52). Infrared spectrometry has been used to quantitate hydrocarbons from an extract using column chromatography (49). Other studies utilized a microdetector for changes in heats of adsorption in the effluent from a liquid chromatograph column to measure amounts of hydrocarbons, and all quantities were determined relative to hydrocarbons in a reference seawater sample (48).

At best, all of the aforementioned studies provide a measure of some portion of the various types of hydrocarbon present in seawater, and extrapolate to the total concentration of hydrocarbons. The limitations of the IR and UV-fluorescence measurements have been previously discussed. Three studies have used column chromatography to isolate hydrocarbons from lipids extracted from seawater and analyzed one or more of the isolated hydrocarbon fractions by gas chromatography (8, 47, 51, 54, 55).

Two of the studies analyzed some of the hydrocarbon fractions by mass spectrometry or GC-MS (47, 43). The mixture of hydrocarbons found in these cases was complex and had a wide molecular weight range. The results for several samples have been interpreted as showing that some of the hydrocarbons are from petroleum sources (54), or are consistent with but do not necessarily indicate petroleum sources (47, 54, 55).

One study analyzed a relatively large number of samples from the western North Atlantic and the Gulf of Mexico (54). There was a considerable geographical variability in the concentrations and composition of hydrocarbons. This variability also was apparent in another study in the eastern Gulf of Mexico (55). The reported concentrations of hydrocarbons have varied within the range 1.0-100 $\mu g\ l^{-1}$ for all of the above studies combined. Squalene and another polyunsaturated hydrocarbon, heneicosahexaene, have been isolated from surface seawater in a coastal region (8). Other studies of hydrocarbons in seawater have not reported the presence of olefins or have presented no definitive data to demonstrate their presence or absence. In several of the studies seawater samples were filtered before analysis of the water and for the rest of the studies they were not. A comparison of the data is hampered by this variable. There are no data available on the concentrations of hydrocarbons on particulate matter and in filtered seawater taken from the same sample.

In most studies there was little attention given to analyzing control samples to ensure that water samples were not contaminated during the sampling and analysis operations, a problem which is particularly hard to avoid for seawater samples collected and fully or partially extracted at sea. It does not suffice to run controls on the analytical procedures and solvents used in a shore-based laboratory if some of the extractions were carried out at sea.

Based on the variability of hydrocarbons among species and subpopulations of marine organisms, and on the biochemical stability of hydrocarbons, Blumer and co-workers suggested that when released to the water column hydrocarbons would serve as unique signatures for the various water masses of the ocean (8, 78). Thus far this hypothesis has not been rigorously tested. The hypothesis has been restated recently (41, 55). It is interesting to note that with the exception of coastal water samples (8) the analyses of water samples previously cited have not allowed unambiguous correlations of hydrocarbon composition with the hydrocarbon composition of phytoplankton and zooplankton living in the water mass. This may be a function of petroleum pollution or hydrocarbons contributed by the plankton, or it may be the result of petroleum hydrocarbon contamination during sampling and analysis.

'Tarballs' and Tar Particles - We offer only a brief synopsis of a recent extensive discussion of this subject (84) and comment on some immediate research needs. The present data on the distributions of tar in the ocean are mainly collected from the western North Atlantic Ocean. There is a need for collecting more extensive data on tar distribution in the world's oceans. Of particular importance is the extension of surveys for tar particles to the waters underlying the surface layers of the ocean. This would establish whether or not tar particles sink from surface waters into the deep waters and are eventually deposited as sediments. Recently, small bits of tar have been observed in a few cores of sediment taken in 1500 m of water in the Gulf of Mexico (85).

Analyses of the chemical composition of tar are for the most part limited to gas chromatography analysis, fractionation by column chromatography, or metal analysis. Thus, there are data available about the molecular weight range or boiling-point distribution of tar constituents and the relative proportions of classes of compounds and metals. The distribution of n-alkanes and branched alkanes has been determined for a large number of tar particles. Detailed analyses of the composition of the cyclic alkanes, aromatic hydrocarbons, and heteroatom compounds are not yet available, but should be forthcoming in the near future as a result of ongoing research.

Chemical analysis of tar particles strongly indicates that the major source of the tar in the western North Atlantic is from tanker ballast discharges (84). Analyses of tar particles in the western Gulf of Mexico suggests that some of the tar particles in this location have an oil-seep source (86).

Slicks - Analyses of hydrocarbons in slicks not associated with known oil-pollution incidents have been reported (87, 88). A comparison of the concentration of hydrocarbons in the surface microlayer and in water 20 cm below the surface taken in coastal waters showed some enrichment in the surface microlayer (87). Identification of individual hydrocarbons was hampered by low concentrations of 5.9-8.5 $\mu g\ l^{-1}$ and small sample size. Several surface microlayer and 20 cm subsurface samples from the North Atlantic have been analyzed (88). The composition of the hydrocarbons was variable and within the molecular weight range of the 16-30 carbon n-alkanes. Concentration values ranged from 5 to 55 $\mu g\ l^{-1}$ for surface microlayer samples and from 10 to 56 $\mu g\ l^{-1}$ for subsurface samples with no consistency of enrichment of hydrocarbons in the surface microlayer.

Surface Sediments - There have been several studies devoted to the analysis of hydrocarbons in ancient sediments and sediments less than 10,000 years old which were buried and not in contact with the contemporary marine environment. The reports in the literature have been reviewed (7, 9, 10).

Analyses of surface sediments in some coastal areas free from petroleum pollution or natural oil seeps have shown that C_{25}, C_{27}, C_{29}, and C_{31} n-alkanes predominate in the higher molecular weight fraction of hydrocarbons. These n-alkanes presumably have their origin in land plants and marsh-grass waxes. Branched alkanes, including the isoprenoid hydrocarbon pristane, have been reported for surface sediments (89). Sever (31) reported some very interesting hydrocarbon distributions for surface sediments in coastal and lagoonal areas of the Gulf of Mexico. She found a wide-ranging distribution of 21-30 carbon n-alkanes with a predominance of odd-carbon n-alkanes, the branched iso- and anteiso-17- and 18-carbon alkanes, and the 7- and 8-methylheptadecanes. She concluded that the composition of the hydrocarbons in these surface sediments pointed towards blue-green algae and higher plants as a primary source for the hydrocarbons.

Surface sediments of a marine lagoon on Surtsey, a recently formed volanic island, contained a hydrocarbon mixture suggesting a blue-green algal origin (60). There was an even distribution of the 17-30 carbon n-alkanes. The isoprenoid hydrocarbons pristane and phytane were reported. Pristane and phytane were also reported in surface sediments from a reducing environment in a coastal region (69) and from surface sediments of the Dead Sea (85). Whereas pristane has been reported to be present in surface sediments from several locations, phytane has not been found in some surface sediments (59, 89).

There are a few reports of the presence of alkenes in surface sediments. In some cases gas-chromatographic analyses of hydrocarbons isolated from sediments have shown that one or two alkenes of undetermined structure are the predominant hydrocarbons in the surface sediments (58, 91, 92). Heneicosahexaene and heptadecene have been reported to be present in surface sediments (92).

The diagenesis of hydrocarbons in a salt marsh environment has been studied by Johnson and Calder (93). They concluded that in situ microbial activity caused major changes in the composition of hydrocarbons, and that these changes occur rapidly and are of large magnitude. The changes appeared to be similar but not exactly the same for both reducing and oxidizing depositional environments. Changes in hydrocarbon composition and concentration in sediment samples from a reducing evironment in another geographical location, and further offshore, were not as marked (59).

The cycloalkanes have not received the attention accorded the n-alkanes and branched alkanes. Their presence in sediments containing polluting petroleum hydrocarbons has been noted by the unresolved complex mixture in the gas chromatograms of the alkanes isolated from the sediments (38, 58, 65, 94, 95).

Aromatic hydrocarbons have also not been subjected to the same extensive study that has been accorded the n-alkanes and branched alkanes. The exceptions to this generalization are 3,4-benzopyrene and perylene. There has been a review of the analyses for 3,4-benzopyrene in marine samples which includes several papers on analyses of surface sediments (96). The dominant method of analysis has been, until recently, separation of a hydrocarbon fraction containing the 3,4-benzopyrene and then analysis of this fraction by UV-fluorescence spectrometry. There are some problems with the discriminatory power of some of these methods of analysis and we think that application of more sophisticated techniques, such as further separation of the 3,4-benzopyrene from other interfering compounds and analysis by mass spectrometry and UV-fluorescence spectrometry, will be needed to provide more accurate concentration values for surface sediment samples.

The reports of perylene in surface sediments have recently been reviewed by Aizenshtat (97). Several mechanisms have been postulated for the chemical formation of perylene from 4,9-dihydroxyperylene-3,10-quinone and from erythroaphin pigments in reducing environments. This is a restatement of an earlier theory based on more extensive analysis. Two recent papers report analyses of aromatic hydrocarbons in surface sediments of the French Coast (73). Concentrations of 3,4-benzopyrene,8,9-benzofluoranthene, perylene, and fluoranthene varied from not detectable to 13.0 μg $(100\ g)^{-1}$ dry weight for each hydrocarbon. In addition, there appeared to be a series of alkylated aromatic hydrocarbons. The more complex mixtures of aromatic hydrocarbons occurred at the places where there was usually a higher concentration. This was interpreted as indicating petroleum pollution in the surface sediments, an interpretation supported by the extreme complexity and wide molecular weight range of the saturated hydrocarbons (73).

Several papers have dealt with the detection of petroleum pollution in surface sediments (38, 39, 58, 65-67, 73, 74, 94, 95). In general, the extreme complexity and wide molecular weight range of hdrocarbons, along with high concentrations of hydrocarbons, have been interpreted to demonstrate petroleum pollution in the sediments. The rationale supporting this interpretation is discussed along with other possibilities which might lead to the complex mixture of hdrocarbons in sediments with a wide molecular weight range (20, 38, 40, 58, 66, 94, 95). A detailed study of hydrocarbons in sediments polluted by a No. 2 fuel oil spill - West Falmouth, Mass. Oil Spill - has been conducted over a two-year period and is to date the most extensive and comprehensive study of the detection of petroleum hydrocarbons in surface sediments and the fate of petroleum hydrocarbons once incorporated into surface sediments (66, 94, 95). Some of the conclusions are as follows. The fuel oil penetrated to a depth not exceeding 7.5 cm. Partial dissolution and bacterial degradation altered the composition of the oil near the sediment-water interface. However, oil was still present in the sediment for two years after the spill and demonstrated that oil products and crude oils can have a considerable environmental persistence.

The persistence of a portion of petroleum hydrocarbons from a spill was also documented for oil coming ashore on a beach at Martha's Vineyard, Mass. and oil coming ashore on Bermuda (98).

Marine Atmosphere - A few samples of the marine atmosphere taken from a tower on Bermuda have been analyzed for higher molecular weight hydrocarbons. Normal alkanes, branched alkanes, and a mixture of hydrocarbons not resolved by medium-resolution gas chromatography, probably cyclic alkanes and branched cyclic alkanes, were reported for the molecular weight range between that of the C_{14} and C_{32} n-alkanes. A review of the scanty information available concerning hydrocarbons in the marine atmosphere was also presented in that paper (99).

Concentrations of Hydrocarbons in Seawater, Organisms, and Sediments - The usual ranges of concentrations of hydrocarbons in the marine environment are given below. We present these in a separate section to facilitate a comparison of the concentration levels in the various types of sample. The values are taken from references cited in the previous sections.

Seawater. Concentrations of 1-100 $\mu g\ l^{-1}$ or 10^{-9} parts per part of total hydrocarbons are usually found. Individual hydrocarbons may be present at the ng l^{-1} level. Higher concentrations have been reported in areas near industrial effluents and municipal sewage effluents. In general, surface waters contain higher concentrations than deeper waters of the ocean.

Tar particle concentrations in the upper 1 m of the water column range from 0 to 40 mg m^{-2} with up to 500 mg m^{-2} reported for some areas of the Mediterranean Sea. Thus, tar particle concentrations in surface waters are of the order of 0-40 $\mu g\ l^{-1}$, which represents a substantial portion of the total hydrocarbon concentration in some surface water samples.

Organisms. Hydrocarbon concentrations in organisms range from 1 to 200 $\mu g\ g^{-1}$ wet weight or 10^{-6} parts per part for total organisms. Higher concentrations are present in certain organs; e.g. shark liver samples have concentrations up to 1300 $\mu g\ g^{-1}$ wet weight. In some cases one or two hydrocarbons account for almost all the hydrocarbon content of a sample.

Sediments. Hydrocarbon concentrations of 1-100 $\mu g\ g^{-1}$ dry weight or 10^{-6} parts per part are found for sediments in the continental margin areas, i.e. continental slope and continental shelf. The sediments in estuarine areas may have higher concentrations but usually range from 5 to 100 $\mu g\ g^{-1}$ dry weight. There are no values available in the literature for surface sediments below the 3500 m water depth.

Oil-polluted Samples. Hydrocarbon concentrations in oil-polluted samples range up to 545 $\mu g\ g^{-1}$ wet weight for organisms in coastal waters and up to 12,400 $\mu g\ g^{-1}$ dry weight for sediments in coastal areas. Concentrations in water samples associated with spills are high, but there is no way of separating the total concentration into a dissolved portion and an emulsified portion. Values of up to 1.0 mg l^{-1} have been reported for oil in water not immediately adjacent to a slick.

Processes Controlling the Distribution of Hydrocarbons

Physical-Chemical. The mechanisms of input into the marine environment - biosynthesis, effluent discharges, accidental oil spills, atmospheric fallout, etc. - have an obvious influence on the distribution of the hydrocarbons. The mechanism of input also determines whether the hydrocarbons enter at the fresh water-salt water interface, the air-sea interface, or the sediment-water interface or are directly introduced as an integral component from organisms. The input processes also determine whether the hydrocarbons enter in a dispersed form or in a cohesive manner such as an oil slick.

Quinn and co-workers (100-102) have recently reported a series of studies on the adsorption-desorption of alkanes, aromatic hydrocarbons, and No. 2 fuel oil on minerals and the solubilization of hydrocarbons in seawater. Hydrocarbons can be removed from marine waters by adsorption on to mineral particles and incorporation into the underlying sediments. This association process can occur by two routes: contact of the hydrocarbons and mineral particles in the upper parts of the water column achieved during the settling of the mineral down past the floating or suspended hydrocarbons, or transport of the hydrocarbon by water circulation to the bottom. There the hydrophobic hydrocarbon can adhere to the bulk sediments. Regardless of the process involved it is unlikely that 100% of the hydrocarbons become associated with the sediments. Many of the hydrocarbons have small but measurable solubilities in water, so that at least a fraction will dissolve in seawater rather than adsorb onto solid surfaces. The differences in relative solubilities of the various types of hydrocarbon - normal alkanes, branched alkanes, aromatic - can lead to the distribution in sediments observed in laboratory and field studies of petroleum-polluted sediments (38, 58, 66, 73, 94, 95). A study of a No. 2 fuel oil spill (66) showed that after two years aromatic hydrocarbons exhibited preferential losses of lower molecular weight components from marine sediments while more highly substituted aromatics, which would be more insoluble, were preferentially retained. Uptake and retention by marine sediments of n-alkanes and branched alkanes was greater than that of relatively more soluble cyclic and aromatic components of petroleum in a study of adsorption of petroleum from water (100, 102).

Several factors can influence the solubility of hydrocarbons in seawater and thus modify their incorporation into sediments. Water temperature and salinity would affect uptake by sediment. The presence of certain types of dissolved organic compounds in seawater can enhance the solubility of hydrocarbons (51, 100). Fulvic acid is an example. Poorly soluble hydrocarbons can associate with these relatively soluble materials and become distributed in seawater in true solution or in finely dispersed micelles. This tends to diminish their adsorption by sediment particles (100-102). Laboratory studies have shown that removal of indigenous organic matter results in a significant increase in the ability of sediments to adsorb hydrocarbons (100, 102). However, because of a high surface area per unit of sediment rising from a small average grain size or clay mineral content, the sediments which have adsorbed more natural organic matter also tend to adsorb more hydrocarbons than other sediments. Thus, a correlation between total organic content and hydrocarbon uptake is observed.

Biological. Hydrocarbons biosynthesized by organisms are released to seawater or sediments by excretion from living organisms or by autolysis and decomposition of dead organsims.

The uptake, retention, and discharge of hydrocarbons by living organisms has received considerable attention in the recent literature because of its importance to the question of biological effects of oil pollution and the potential public health hazard posed by oil-polluted marine food which might be consumed by man. The earlier work of Blumer and co-workers suggested that hydrocarbons, particularly pristane, were passed up the food web from prey to predator (20, 78). Analysis of oil-polluted shellfish before and after placing the shellfish in clean seawater in a laboratory showed very little loss of the petroleum hydrocarbons after at least 60 days (65).

Four recent studies (22, 103-105) have demonstrated that there is an active uptake of hydrocarbons from seawater across the membrane system of shellfish and fish. Presumably the primary site of uptake is the gill membranes based on studies using ^{14}C-labelled hydrocarbons (22). Within marine organisms there are varying concentrations of hydrocarbons in tissues and organs. Lipid-rich organs such as the liver and reproductive organs have higher concentrations of hydrocarbons than relatively lipid-poor tissues such as muscles (23).

The composition of petroleum hydrocarbons in animals exposed to No. 2 fuel oil was definitely different than the hydrocarbon composition of the No. 2 fuel oil in several studies (65, 103-105). Aromatic hydrocarbons were taken up to a greater extent than were saturated hydrocarbons (103-105). One study showed the opposite (22), a result which may be linked with the fact that the organisms were not simultaneously exposed to both saturated hydrocarbons and aromatic hydrocarbons.

The studies (22, 103-105) also showed that petroleum hydrocarbons taken up by shellfish were discharged to a significant extent when placed in clean water for periods of up to 52 days. However, in some cases there were still detectable levels of petroleum hydrocarbons after several weeks of exposure to clean water (22, 103). It is obvious that several processes and factors are operative in the relationship between hydrocarbons in organisms and hydrocarbons in their surrounding habitats. The findings of Blumer and co-workers are not necessarily in contradiction to the findings of the other workers. The former studied shellfish which had been exposed over a two month period to a massive dose of oil in their habitat from an oil spill whereas the latter exposed shellfish to soluble or 'accommodated' oil in seawater in laboratory experiments. Stegeman and Teal (103) have summarized all of these studies in their statement, 'We suggest that the concentration and composition of non-biogenic hydrocarbons in oysters reflects both the complete exposure history and the current exposure level, and results from the interplay between uptake, release, retention, route of entry, and possible residence of hydrocarbons in multiple (lipid) compartments'!

Clearly, this is an area of active research with the promise of some significant advances in our understanding of the relationship between organic compounds in seawater, their uptake by, retention by, effect on, and release by marine organisms.

5. Fate of Hydrocarbons in the Marine Environment

There are several mechanisms for the removal of hydrocarbons from the contemporary marine environment. These are photochemical and/or chemical alteration to other compounds, biochemical alteration to other compounds, transfer to the atmosphere, or burial in sediments as geological deposits. The relative importance of the processes depends on the hydrocarbon or mixture of hydrocarbons in question. The lower molecular weight hydrocarbons (C_1-C_{10}) would be influenced to a greater extent by evaporation and solubilization processes. Photo-oxidation would be expected to have a greater effect on aromatic hydrocarbons and heteroatom aromatics than on n-alkanes and branched alkanes, although the photochemistry of complex mixtures of organics is a difficult process to predict. Although auto-oxidation is certainly to be expected in spilled oil, its relative rate may be much slower than the other processes acting on the oil.

Incorporation into Sediments - Incorporation into sediments by mixing processes, deposition of hydrocarbons adsorbed onto particulate matter or incorporated into particulate matter, is one fate of hydrocarbons in the marine environment. The processes have been discussed earlier. Biochemical degradation of the hydrocarbons after incorporation into sediments may be much slower than in the water column or at the air-sea interface because of lower oxygen concentrations or anaerobic conditions in the sediments (66, 44, 95). Hydrocarbons surviving the degradation process in the surface sediments are eventually buried and become geochemical deposits. Studies of the similarities in chemical structure between hydrocarbons found in ancient sediments and hydrocarbons synthesized by organism offer strong evidence that hydrocarbons survive for millions of years once incorporated into deeply buried sediments (7, 9, 10).

Transfer to the Atmosphere - Hydrocarbons from oil slicks and hydrocarbons from biosynthetic processes incorporated into natural sea slicks may enter the atmosphere by evaporation or by injection during turbulence, or via bubbles bursting through the surface film (87, 88, 106, 107). There has been little published on this particular fate. Once in the atmosphere the relative amounts that are chemically or photochemically degraded and the amount which is redeposited onto the oceans or land are unknown.

Biochemical Alteration - The microbial degradation of oil pollutants and specifically hydrocarbons in the marine environment has been recently reviewed (108). Earlier reviews of petroleum microbiology and microbial degradation of oil in the marine environment are also recommended (109-110). In general, studies have shown that n-alkanes are more easily degraded than are the other types of hydrocarbon. Branched alkanes are the next in order of ease of biodegradation. Cyclic alkanes and aromatic hydrocarbons seem to be much more resistant to biochemical degradation by microorganisms.

A two-year study of petroleum hydrocarbons incorporated into sediments from an oil spill has demonstrated that the above order of biochemical degradation is the same in a natural marine system (66, 95).

It has been established that the ability of microorganisms to degrade hydrocarbons depends on several factors including temperature, pH, nutrients, presence of other organic compounds, previous exposure of the culture to various types of hydrocarbon, and physical agitation or mixing. Detailed studies of the microbial degradation of hydrocarbons, particularly cyclic alkanes and aromatic hydrocarbons, in natural systems are needed to further our understanding of the processes and rates of degradation of hydrocarbons in seawater and sediments.

Bacteria capable of partially degrading petroleum have been isolated from several locations in the world's oceans (108, 111). However, the rate at which hydrocarbons are degraded in the open ocean has not yet been ascertained.

The products of biochemical degradation of hydrocarbons have been recently summarized. The principal products resulting from the microbial degradation of hydrocarbons are various hydroperoxides, alcohols, phenols, carbonyls, aldehydes, ketones, and esters in addition to CO_2 and H_2O (108). The exact pathways of biochemical degradation are poorly understood.

There are a few reports of studies of the metabolism of hydrocarbons by marine animals - both fish and invertebrates - using radioisotope-labelled hydrocarbons (22, 112). Animals were exposed to seawater containing the labelled hydrocarbons. Thus, the studies did not deal with metabolism of hydrocarbons introduced via feeding but rather those taken up across body membrane surfaces such as the gills. It was demonstrated that certain fish can metabolize the aromatic hydrocarbons naphthalene and 3,4-benzopyrene, with the major metabolites tentatively identified as 7,8-dihydro-7,8-dihydroxybenzopyrene and 1,2-dihydro-1,2-dihydroxynaphthalene. However, the marine mussel *Mytilus edulis* did not metabolize n-heptadecane, tetrahydronaphthalene, naphthalene, or 3,4-benzopyrene when tested under similar conditions (112). The studies have been thus far limited in the length of time of observation and the species investigated. Nevertheless, they offer a model for more extensive investigations.

6. Oil Spills

The fates of actual and simulated oil spills have been studied with varying degrees of scientific sophistication. The exact fate of each spill is unique since it is influenced by several environmental parameters such as temperature, salinity, wind and wave conditions, suspended sediment load, and geographical location and by the chemical composition of the spilled oil (19, 98, 113). The studies as a whole point to the following processes as important influences on the fate of oil: dissolution, evaporation, photo-oxidation, adsorption onto particulate matter, mixing below the surface, which often includes mixing into sediments in shallow areas, adsorption onto rocky shores and beaches, and microbial attack and degradation (19, 98).

Gruenfeld (114) and Zafiriou et al. (115) among others, have recently reviewed the principles of passive tagging or fingerprinting of petroleum contamination - the correlation of a particular case of petroleum contamination with its source or sources of contamination by primarily chemical parameters.

There is extensive literature on the subject of oil spills, oil pollution and its effects and fate. Several compendiums of references are available (116-118). For those readers who wish a detailed listing of all literature dealing with oil pollution, references 119 and 120 are suggested. A recent study sponsored by the U. S. National Academy of Sciences concerning the 'Input, Fate and Effect of Oil in the Marine Environment' should be issued by the time this report is published (120).

7. Summary

The study of hydrocarbons in the marine environment is now progressing at a faster pace owing to the interest in understanding the inputs, fate, and effects of oil pollution. However, it is clear that a more complete knowledge of the hydrocarbons introduced into the marine environment by natural processes such as biosynthesis is needed.

All studies of hydrocarbons are dependent on the analytical techniques employed, and our knowledge of hydrocarbons in the marine environment was, until recently, hampered by a lack of a more widespread application of sophisticated methods of analysis. Because of their ease of analysis and their predominance in most marine samples, n-alkanes, n-alkenes, and branched alkanes have been studied to greater extent than other types of hydrocarbons. A more detailed knowledge of the cyclic and branched cyclic alkanes and naphthenoaromatic and aromatic hydrocarbons in marine samples is essential. Many of the important questions relating to oil-pollution studies and to chemical processes in surface sediments will be answered by such knowledge.

There have been significant advances in the past few years in understanding the interactions between hydrocarbons and minerals, and dissolved organic matter and hydrocarbons. These studies, when expanded and coupled with similar studies of other organic compounds in the marine environment, will provide us with a more detailed knowledge of the processes governing the distribution of organic compounds.

The chemical and biological processes acting on hydrocarbons in surface sediments are being explored. Concurrently, the role of sediment microorganisms as a source for hydrocarbons in surface sediments is being mentioned with increasing frequency. Only a few studies have demonstrated that microorganisms contribute substantially to the hydrocarbons in surface sediments. We expect that more evidence will be forthcoming in the near future. We also expect that during the next few years it will become more apparent that chemical and biological processes are operating in surface sediments and have a significant influence on which types of organic compounds are found in ancient sediments.

There is a dynamic and complicated interaction between hydrocarbons in organisms and hydrocarbons in the surrounding environment. Furthermore, key studies have established a relationship between concentration and composition of hydrocarbons in organisms and the growth stage or stage of tissue development. The door to understanding the biochemical role of hydrocarbons has begun to open, with great promise for significant advances in this area of research. Concurrently, the pathways of biosynthesis of hydrocarbons will be unraveled.

There has been a significant advance in our understanding of the physical, chemical, and biological processes affecting oil spilled into the marine environment particularly with respect to the biodegradation of oil by microorganisms. This knowledge is far from complete, and we cannot predict with certainty the fate of any given oil spill. However, it is certain that petroleum hydrocarbons can and do linger long after visual evidence of the oil spill has disappeared.

SECTION 5

COMPARISON OF SOME METHODS OF ANALYSIS FOR HYDROCARBONS IN CLAMS

1. Introduction

We have investigated the comparative efficiencies of three extraction procedures which have been employed in the study of oil pollution of marine organisms: Soxhlet extraction with 1:1 methanol:benzene; Virtis homogenization with n-pentane and anhydrous Na_2SO_4; and digestion with methanolic KOH. These three extraction procedures were applied to subsamples of a homogenate of hard shell clam (*Mercenaria mercenaria*) tissues and body fluids. We have reported our initial results previously (4).

The digestion procedure was applied to clams from three different locations: (i) a highly contaminated harbor area, the Providence River flowing into Narragansett Bay, Rhode Island; (ii) a location in the west passage of Narragansett Bay which is not as heavily contaminated as the first area, (iii) a relatively clean cove area of Waquoit Bay near Washburn's Island in the Town of Falmouth, Cape Cod, Massachusetts. Clams from the first two locations were analyzed for hydrocarbons in 1970 (57) and our analyses of 1973 samples serve as a progress report on petroleum contamination at these locations.

We have also investigated the effectiveness of the digestion procedure followed by column chromatography and gas chromatography and UV-fluorescence analysis for quantitative and qualitative detection of No. 2 fuel oil spiked into clam homogenate subsamples. These experiments evaluate the degree to which varying levels of hydrocarbon concentrations and hydrocarbon compositions already present in shellfish interfere with analyses for the incorporation of recently spilled oil. These experiments have also provided a preliminary evaluation of the limits of gas chromatographic and UV-fluorescence approaches to passive tagging of spilled oil incorporated into organisms.

2. Experimental Methods

Hardshell clams were dredged in November, 1973 from the Providence River at station E just south of Providence, Rhode Island and from station C in the West Passage of Narragansett Bay south of Quonset Point Naval Air Station (see reference 57 for locations). Clams were obtained in June and July of 1974 by hand raking in shallow water of one meter depth near Washburn's Island, Waquoit Bay, Falmouth, Massachusetts.

The outsides of the clams' shells were thoroughly washed in the laboratory with tap water and then distilled water, and then shucked using clean, stainless steel knives. Care was taken to prevent the animals from being contaminated during shucking and to collect as much of the liquid inside the shell as possible.

Tissue and fluid batches of about 400 g were homogenized at high speed in a Virtis Model 23 homogenizer to a mixture which appeared homogeneous to visual inspection. The homogenate was stored at 0° to 4° until use.

Extraction by Virtis Homogenization - Fifteen grams of clam homogenate, 100 g of Na_2SO_4 and 150 ml of n-pentane were added to a 200 ml Virtis flask. The contents of the flask were blended with a glass stirring rod and then mixed in a Virtis homogenizer for exactly two minutes at high speed. The pentane extract was decanted onto a column containing 100 g Na_2SO_4 and allowed to percolate over the column into a collecting flask. The tissue was extracted two more times in this manner before the entire contents of the flask were charged to the Na_2SO_4 column. The column was eluted with n-pentane until the eluate was clear and colorless. This usually required about 200 ml of n-pentane.

When 50 to 60 g tissue samples were extracted, as many as 5 Virtis runs with 150 ml of pentane each were required to extract the same amount of hydrocarbons as above on a weight to weight basis.

Soxhlet Extraction - Fifteen grams of clam homogenate were added directly to a pre-extracted Soxhlet cup. The homogenate was extracted with 300 ml of 1:1 methanol:benzene for 24 hours. The extract was removed and stored; fresh solvent was added, and the extraction was continued another 24 hours. The extracts were combined, and the lipids were partitioned into n-pentane. When 50 g of starting material was used, the extraction was run with two 600 ml batches of solvent in larger Soxhlet extractors.

Digestion Extraction - A mixture consisting of 50 g clam homogenate and a volume of 75 or 150 ml of 6.7% KOH in MeOH was refluxed for one hour. The digestion method, unlike the two methods described above, accomplishes both extraction and saponification of lipids in one step. The non-saponifiable lipids were then partitioned into n-pentane. A second digestion procedure using KOH in methanol:benzene (1:1) using the procedure described was also tested.

Saponification of Lipids and Isolation of Hydrocarbons - Extracts obtained by the Virtis and Soxhlet methods were saponified under reflux in 250 ml of 0.4 N KOH in methanol:H_2O 3:1 for 24 hours. Non-saponifiable lipids were partitioned from the aqueous-methanol solution into pentane. Column chromatography of extracts from the Virtis, Soxhlet and digestion methods was accomplished using columns of 1:1 alumina packed over silica. The alumina and silica were activated overnight at 250°C and 120°C respectively, and then both were deactivated with 5% water. The ratio of column material to non-saponifiable lipids was 100:1 or greater. Extracts were charged to the columns in small volumes of n-pentane and then eluted with one column volume each of (a) n-pentane, (b) 10% benzene in pentane,

(c) 20% benzene in n-pentane, and (d) benzene. The n-pentane elution will be referred to in subsequent discussions as fraction one (f_1). The second and third elutions were combined for subsequent analysis and will be designated as fraction two (f_2), and the benzene elution designated as fraction three (f_3). The three fractions, f_1, f_2, and f_3 contain as predominant compounds alkanes, cycloalkanes, and mono- and diolefins; polyolefins and one and two ring aromatic hydrocarbons; and three- to six-ring aromatic hydrocarbons respectively as determined by studies of mixtures of known compounds.

Aliquots from the three fractions were weighed on a Cahn electrobalance before gas chromatographic analysis.

Gas Chromatography - Two columns were employed in these studies. The first, 15.2 meters, 25,000 theoretical plate OV-101 SCOT column (Perkin-Elmer Corporation) was programmed from 70°C to 270°C at 6°/minute with a He carrier gas flow rate of 3 ml/minute. The second column was a 3.1 meter x 3.2 mm o.d. 3% SE-30 on 100/120 chromosorb W programmed at 6°/minute from 60°C to 270°C with a He carrier gas flow rate of 6 ml/minute. The instruments employed were Varian Model 1400 and Model 2700 gas chromatographs equipped with flame ionization detectors.

Fluorescence Spectra - Column fraction 1 is not generally useful in fluorescence analysis since it contains no fluorescing compounds in Kuwait crude co-chromatograph with the saturates in fraction 1. This also occurs for the station E extract but does not occur for API No. 2 fuel oil or the other clam extracts. Fractions 2 and 3 contain the preponderance of strong emitters. In this study we focus on fraction 2 for two reasons: (a) the emitters in the API No. 2 fuel oil we have used for spiking elute primarily in fraction 2 and (b) fraction 3 weights and compositions were not reproducible so comparison between extracts is very difficult.

All spectra were obtained on a Perkin-Elmer MPF-3L UV-fluorescence spectrophotometer. Scans were run at 30 nm/minute in the instrument's direct mode. Except when the low temperature accessory was in use, all solutions were run in 1 cm fused silica cells.

Solutions were prepared to be as dilute as possible in order to minimize concentration quenching effects. Concentrations of about 2 ppm in methylcyclohexane solution were usually sufficient to produce good quality spectra of fraction 2 hydrocarbons. Single distillation of technical grade MCH through a 600 x 20 mm fractionation column sufficiently cleaned up the solvent for our use. This procedure failed to remove a contaminant (probably an alkylated benzene) which produced a single emission bond at 300 nm when excited at 277 nm.

After every sample spectrum was reworked, a spectrum of the solvent was recorded under the same conditions. Most spectra were run at high enough sensitivity that a significant Raman line due to symmetric C-H stretching in the solvent appeared at 316 nm (λ_{ex} = 290 nm), 33% nm (λ_{ex} = 310 nm) and 377 nm (λ_{ex} = 334 nm).

Emission spectra of every extract were run with the excitation monocromator set at 290 nm, 310 nm, and 340 nm. Two of the excitation wavelengths, 290 nm and 340 nm, have been reported to be useful for identification of bulk oil samples cooled to 77°K (127). Excitation at 310 nm has been used extensively in fluorescence studies of hydrocarbons in seawater (127). We found that excitation at or near 290 nm in most cases produced the principal fluorescence emission spectrum in the samples we examined. Excitation at 340 nm was found to be only marginally useful except when the sample was cooled to 77°K.

Low temperature spectra were run with the Perkin-Elmer phosphorescence accessory in place with the chopper removed.

Solvent and Reagent Blanks - Except as specified all solvents used in this study were redistilled in our laboratory. The anhydrous Na_2SO_4 was Soxhlet extracted with 1:1 methanol:benzene and then dried in an oven prior to use. Procedural blanks employing all steps of the extraction and analytical methods were run at least in duplicate. There was no interference with the analysis of the clam homogenates by hydrocarbons in the blanks.

3. Results and Discussion

Comparison of Extraction Efficiencies - Summarized in Table 1, our comparison of the three extraction methods using station C clam homogenate indicates that these techniques as we have applied them, are of approximately equal efficiency for extracting fraction 1 hydrocarbons and fraction 2 hydrocarbons. The similarity of the gas chromatograms in Figure 5 demonstrates that no one method extracts more efficiently than the others in any particular segment of the n-C_{14} to n-C_{30} molecular weight

TABLE 1. COMPARISON OF EFFICIENCIES OF THREE METHODS OF EXTRACTION OF HYDROCARBONS FROM HARD SHELL CLAMS (MERCENARIA MERCENARIA).

METHOD	µg HYDROCARBONS/g WET WT. CLAM	
	$f_1{}^i$	$f_2{}^i$
Virtis Homogenization with C_hH_{12} (5 analyses)	6.59 ± 1.23	2.07 ± 1.05
Soxhlet: 48 hours with CH_3OH:benzene (6 analyses)	8.74 ± 1.00	3.92 ± 0.31
Digestion in KOH:CH_3OH (6 analyses)	6.57 ± 0.84	3.00 ± 0.41

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[i] See text for hydrocarbons in each fraction.

range for fraction 1 hydrocarbons. Similarly, Figure 6 demonstrates the qualitative similarity of fraction 2 hydrocarbons extracted by the Virtis and digestion methods. We do not have a gas chromatogram of the fraction 2 hydrocarbons from the Soxhlet extraction at this time.

We have also found that certain experimental conditions could be altered without changing the efficiency of a particular method. Soxhlet extraction with methanol was found to be as efficient as Soxhlet extraction with 1:1 methanol:benzene. Digestion with 1:1 methanol:benzene and 0.5 N KOH was found to be no more efficient than digestion with 0.5 N KOH in methanol.

There is statistically significant difference at the 95 confidence level when comparing concentrations of hydrocarbons determined by extraction with the following:

Virtis vs. Soxhlet and Digestion vs. Soxhlet (f_1 and f_2 Table 1). Soxhlet extraction yields slightly more hydrocarbons. Such differences should be taken into account when comparing studies where small differences in total hydrocarbon concentrations are important. For most oil pollution studies to date, the small differences noted, whole, real, would not be significant compared to the differences measured between sampling locations. We will have a more comprehensive data base to demonstrate that the differences may be of practical significance when we have completed the quantitative gas chromatographic analyses.

We have Soxhlet extracted the Na_2SO_4-tissue homogenate mixture from the Virtis extraction procedure to determine whether successive extraction procedures would yield significantly more material. The experiment was run in duplicate. The procedure extracted another 3 μg/g wet weight hydrocarbons in f_1 and 1 μg/g wet weight hydrocarbons in f_2. The additional hydrocarbons extracted had a composition similar to that extracted by the Virtis procedure. It is possible that continued extraction with pentane using the Virtis procedure or additional applications of the digestion procedure would have yielded a similar result. We have not tested all the possible conbinations of successive extractions. The extraction procedures as we have employed them and as they are generally applied according to the literature extract the bulk of the hydrocarbon material from the clam tissue matrix. Additional successive applications of these procedures would probably extract successively smaller amounts of material but would not significantly increase, within the precision of the methods, the determined value for the concentration of hydrocarbons in the clam tissue.

Our results do not support claims by other investigators (42, 104) that the digestion method is considerably more efficient than Soxhlet extraction. This discrepancy would be due to the fact that we homogenized our samples prior to extraction. However, based on our experience, we favor the digestion method for future work. The Soxhlet extraction method requires specialized glassware and extraction periods of 24 to 48 hours. Virtis extraction requires the additional saponification step after initial extraction as does the Soxhlet extraction procedure. In contrast, the

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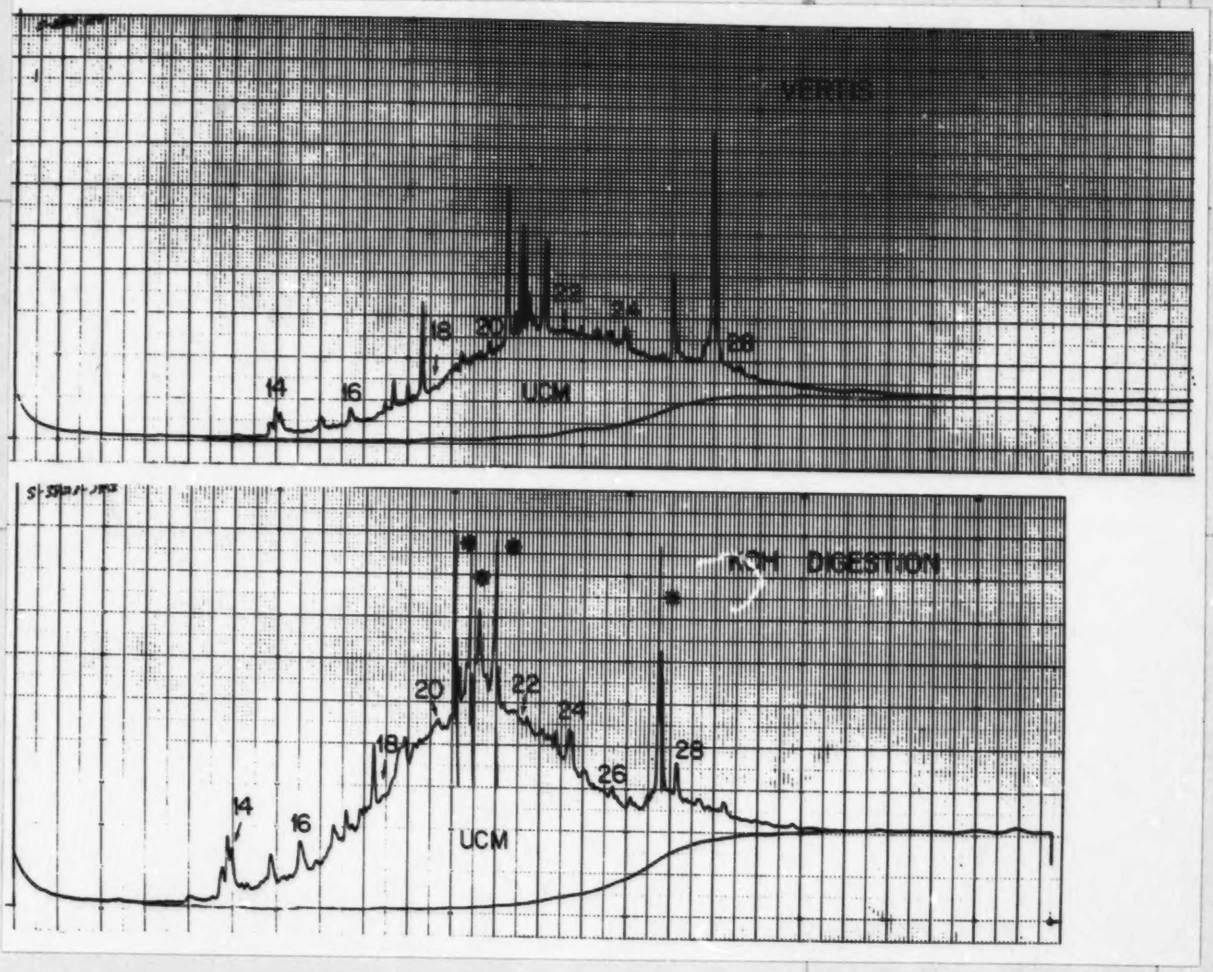

Figure 6. Gas chromatograms of Vertis and KOH digestion extraction.

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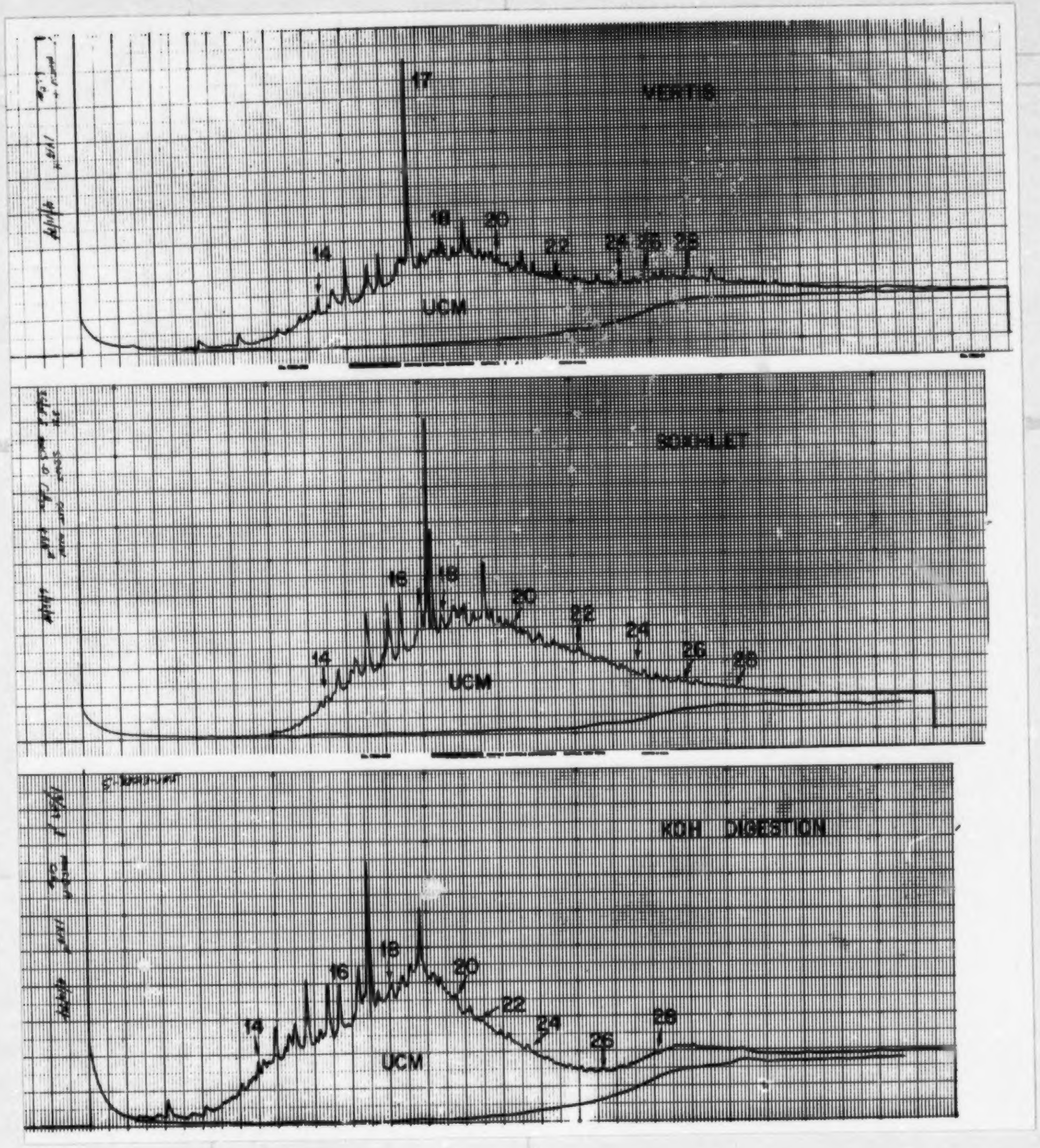

Figure 5. Gas chromatograms of Soxhlet, KOH digestion, and Vertis extracted alkanes and cycloalkanes from clams.

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digestion procedure extracts and saponifies in one step; thus, sample handling is less than for the other procedures. Glassware requirements are minimal. In general, the simplicity of the method recommends it for routine use.

A possible drawback to the digestion procedure is its incompatibility with simultaneous determination of hydrocarbons and chlorinated hydrocarbons of the DDT family in the same extract. The digestion procedure would chemically alter distribution of certain DDT metabolites as they are present in nature.

Thus far studies have focused on extraction and analysis of shellfish. This is reasonable in view of the commercial value of shellfish and their susceptibility to oil pollution as demonstrated by several studies (57, 62, 63). We think it would be of interest to conduct similar tests for other marine organisms since tissue and organ matrices may be such as to alter the extraction efficiency of one or more of the procedures. Also, it is of interest to test the extraction procedures for different concentrations of hydrocarbons. Thus far we have tested them for only one concentration level in shellfish.

Comparison of Hydrocarbons in Clams from Three Areas with Different Petroleum Contamination Levels - Each of the three stations we sampled to obtain clams for our studies are typical of a situation which might exist before an oil spill in coastal waters and thus provides information on baseline hydrocarbon concentration and composition.

Station E (57) is located at the mouth of the Providence River south of Providence, Rhode Island. It is within a few kilometers of two sewage treatment plant effluents in an area of high sedimentation and heavy ship traffic. Shellfishing is prohibited for reasons of public health, although there is a substantial population of clams. Analysis of clams from this location three years ago have hydrocarbon concentrations of 15 µg/g wet weight (57). The complexity and the wide molecular weight range of the hydrocarbon mixture strongly indicated petroleum contamination of the shellfish. Further studies of sediments at this location by Zafiriou (37) confirmed this finding.

Our recent analyses of clams from this location show a four-fold increase (Table 2) in hydrocarbon concentration. The source of the increase, however, is not apparent from our data. If the source were a spill of a fuel oil in the immediate vicinity within two months of our sampling data, then we would expect to find some predominant hydrocarbon peaks in our gas chromatograms (Figure 7) as demonstrated by studies of other spills. We find, instead, an unresolved envelope spanning a wide molecular weight range. The lack of predominant peaks suggests that the source may have been a spilled oil which by the time of our sampling had been weathered and biodegraded to the extent that the n-alkanes and branched alkanes responsible for most of the resolved prominent peaks in gas chromatograms were not present (57). Alternatively, slow dribbling of oil into this area from frequent spills or sewage effluents (14) could have given a similar increase in total concentration. We are aware of only one

TABLE 2. HYDROCARBON CONCENTRATIONS IN CLAMS AT THREE LOCATIONS: RESULTS OF DIGESTION EXTRACTION.

LOCATION	g HYDROCARBONS/g WET WT. CLAM	
	f_1	f_2
Station E - Providence	70.4	17.3
River Narragansett Bay (2 analyses)	66.7	24.9
Station C - Narragansett Bay (7 analyses)	6.57 ± 0.84	3.00 ± .41
Washburn's Island, Falmouth, MA June (2 analyses)	0.51	6.80
	0.40	6.79
Ave.	0.45	6.79
July (4 analyses)	1.88	1.83
	1.69	1.95
	0.95	1.88
	1.35	2.15
Ave.	1.46	1.95

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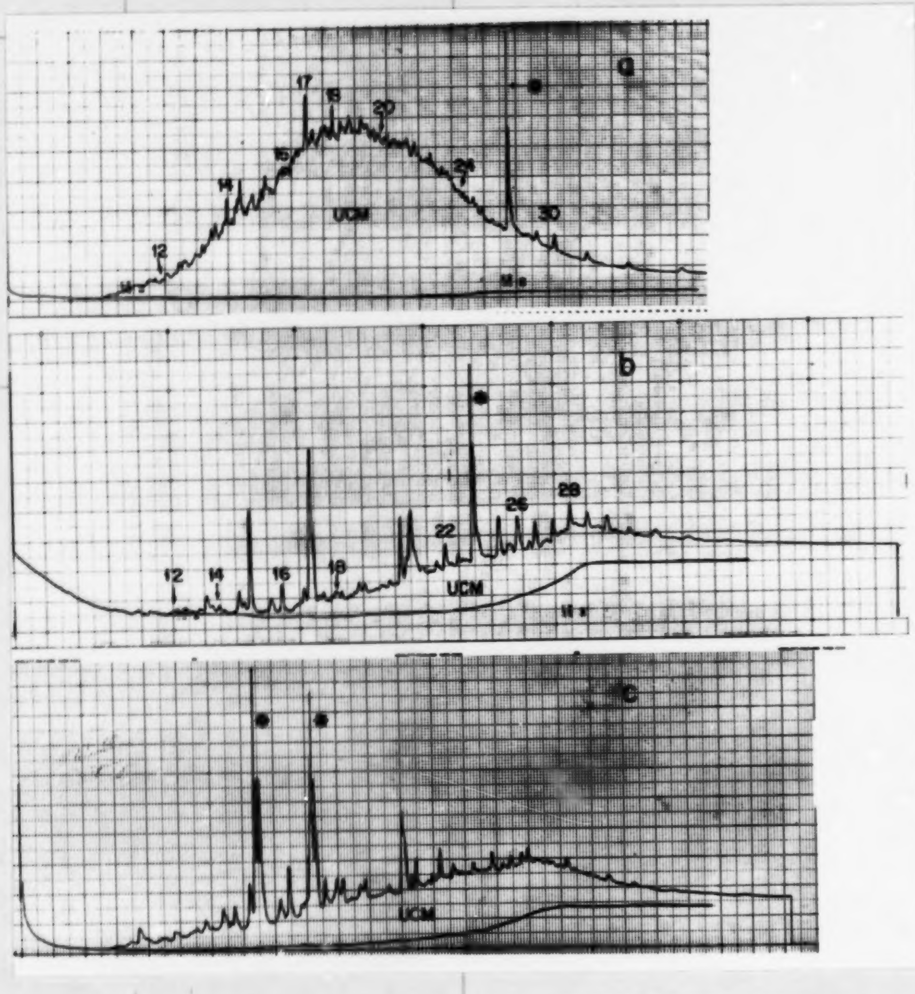

Figure 7. Gas chromatograms of alkanes and cycloalkanes from clams at Station E, Providence River (a), Narragansett Bay (b), and Washburn's Island (c), Waquoit, Cape Cod.

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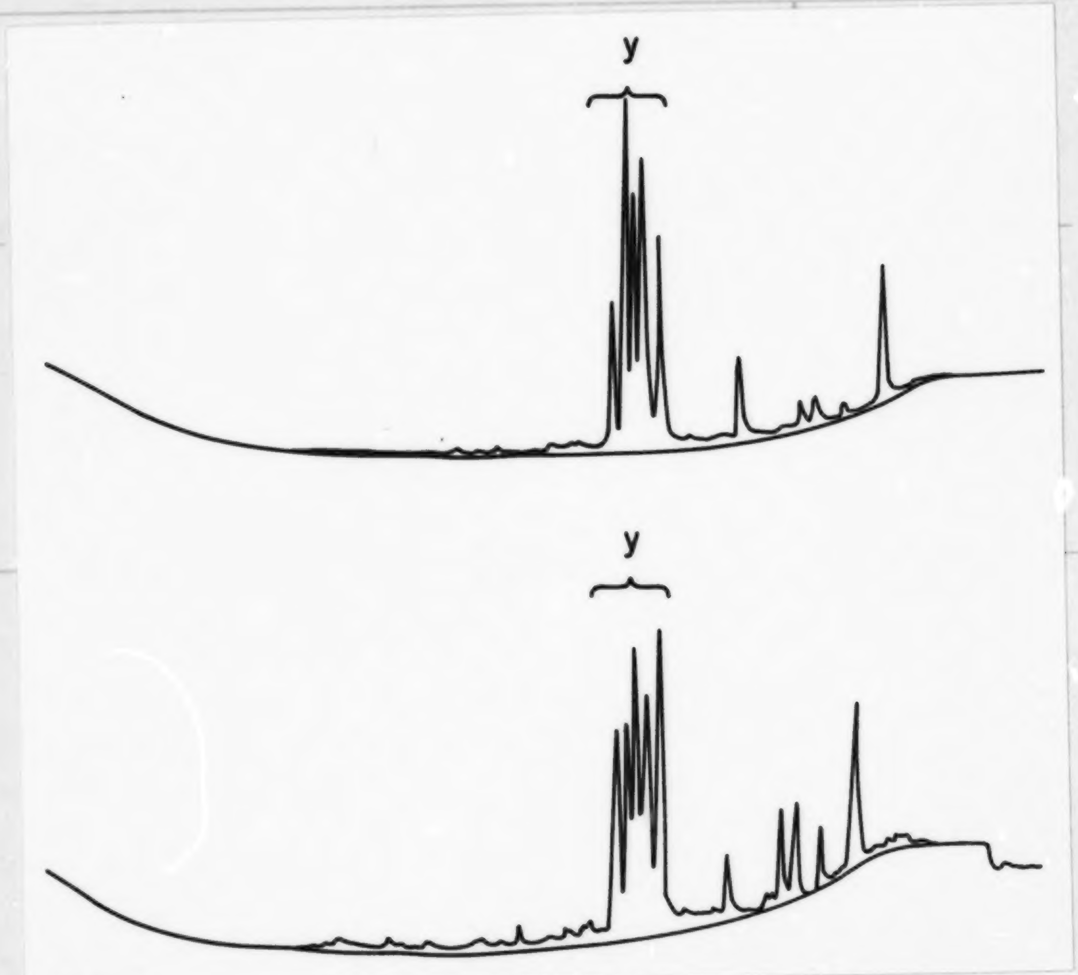

Figure 8. Gas chromatograms of f_2 aromatic hydrocarbon fraction of clams from Washburn's Island, Cape Cod (duplicate analysis) (y = alkenes of biosynthetic origin or geochemical origin).

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serious, large spill near this location: a discharge of 378,429.5 liters of a No. 6 fuel oil on April 9, 1973, six months before we sampled the clam population in the area.

Station C (57) located 4 kilometers south of Quonset Point Naval Air Station in the west passage of Narragansett Bay, Rhode Island, has a shellfish bed of commercial value. Three years ago clams from this area had hydrocarbon concentrations of 10 μg/g wet weight with a complexity and molecular weight distribution indicating that most hydrocarbons were from petroleum contamination. It was concluded at that time that the most likely source of the petroleum contamination was small oil spills and petroleum pollutants transported into the area from other areas such as station E.

The hydrocarbon cencentrations we have measured in station C clams three years hence (Table 2) is slightly lower. Our gas chromatograms do indicate, however, that there is still a very complex mixture of hydrocarbons with a wide molecular weight range which indicates that most of the hydrocarbons are of petroleum origin. Whether or not the decrease in concentration of the hydrocarbons is significant and will continue can only be determined by future measurements. The nearby Quonset Point Naval Air Station has been closed and aircraft carriers no longer visit there. Annecdotal evidence (138) shows that there were several small spills associated with aircraft carrier operations during 1960-1971. While recent research (102-104) indicates that in laboratory studies shellfish reduce their body burden of petroleum contamination as the petroleum concentration in surrounding waters is reduced, it has not yet been demonstrated that this occurs "in situ."

Washburn's Island is located in Waquoit Bay, Falmouth, Massachusetts where ship traffic is limited to pleasure craft mainly during the summer months. The shores of the bay are sparsely populated compared to shore areas near stations E and C in Narragansett Bay. The results of analyses of clams from this area are present in Table 2 and Figures 7 and 8. The concentrations of hydrocarbons are about an order of magnitude less than the concentrations at station C and the qualitative nature and simplicity of the hydrocarbon mixture is consistent with the data of Blumer et al. (63) for scallops taken from Waquoit Bay and analyzed in 1969. The small unresolved complex mixture signal in our gas chromatograms may indicate a very low level of petroleum hydrocarbon contamination, perhaps from pleasure craft exhausts. More detailed analysis of a larger sample of clam homogenate from this region would be needed to verify that petroleum contamination does indeed exist in these clams.

Our data indicate that Waquoit Bay has remained relatively free of petroleum contamination during the past five years.

We note a difference between hydrocarbon concentrations and compositon found in clams sampled in June and in July as indicated in Table 2 and Figure 7. The cause of this difference is not known for certain at this time. Seasonal changes in the hydrocarbon composition of the clam's food or in physiology and biochemistry of the clams themselves are two factors likely to influence the hydrocarbon composition of the clams as demonstrated by studies of algae and other marine organisms (33, 34). The data does reinforce earlier studies which have shown that the concentrations and composition of biogenic hydrocarbons can fluctuate within as yet unknown limits, a factor which should be taken into account when baseline data is obtained for petroleum pollution monitoring.

Spiking Experiments with API No. 2 Fuel Oil - Subsamples of station C and Washburn's Island clam homogenates were spiked at concentrations of 10 ppm (10 μg/g wet weight) and 1 ppm with API No. 2 fuel oil (139). Our aim was to determine the extent to which biogenic and pollutant hydrocarbons already in the clams would interfere with the detection and quantification of newly incorporated oil from a recent spill. This is, of course, a highly simplified model in which physical and biochemical changes in the spilled oil after spillage and during and after incorporation into organisms are not operative. Nevertheless, if the analytical procedure is foiled in the simple test, it will not be of use in the more difficult circumstances of an actual oil pollution incident.

Table 3 gives the quantitative results of the spiking experiments. The average recoveries of spiked material from each fraction and the average total recovery agree very closely with the average recovery of No. 2 fuel oil from our column chromatography procedure. In both cases the loss of roughly 30 of the fuel oil is believed to be from the loss of the more volatile components of the No. 2 fuel oil during the evaporation and concentration procedure. Chromatograms of a spiked f_1 (Figure 9) and an f_1 cut of the No. 2 fuel oil (Figure 10) are depleted at the lower molecular weight end of the fuel oil. No further alteration by our procedures to the composition of the oil is indicated by our data.

Figure 9 contains a series of gas chromatograms of fraction 1 hydrocarbons from (a) unspiked, (b) spiked at 1 ppm, and (c) spiked at 10 ppm station C clam homogenate. The 1 ppm spike is not evident at first glance; however, enhancement of several peaks, particularly the peak due to n-pentadecane (n-C_{15}) is detectable upon close inspection. Clearly, it would be difficult to identify by GC even undegraded No. 2 fuel oil as a pollutant at 1 ppm in these clams when the background level is on the order of 10 ppm hydrocarbons with a complexity and molecular weight range similar to that found in the station C clams. A further implication is that since the hydrocarbon concentration at station E is about ten times greater than at station C, a 10 ppm spike would be just as well masked at station E as a 1 ppm spiked is masked at station C. However, for Washburn's Island clams, a 10 ppm spike is easily identifiable (Figures 10 and 11) due to the low background hydrocarbon concentrations and the predominantly biogenic source and, thus, simple composition for background hydrocarbons.

Figure 9 clearly shows the presence of No. 2 fuel oil at the 10 ppm level. However, unambiguous assignment of the source of the oil cannot be made using the gas chromatographic passive tagging approach employed for oil samples (126). We have calculated ratios of n-heptadecane (n-C_{17})/pristane: n-octadecane (n-C_{18})/phytane; pristane/phytane; and n-C_{17}/n-C_{18} for the API No. 2 fuel oil, the f_1 column

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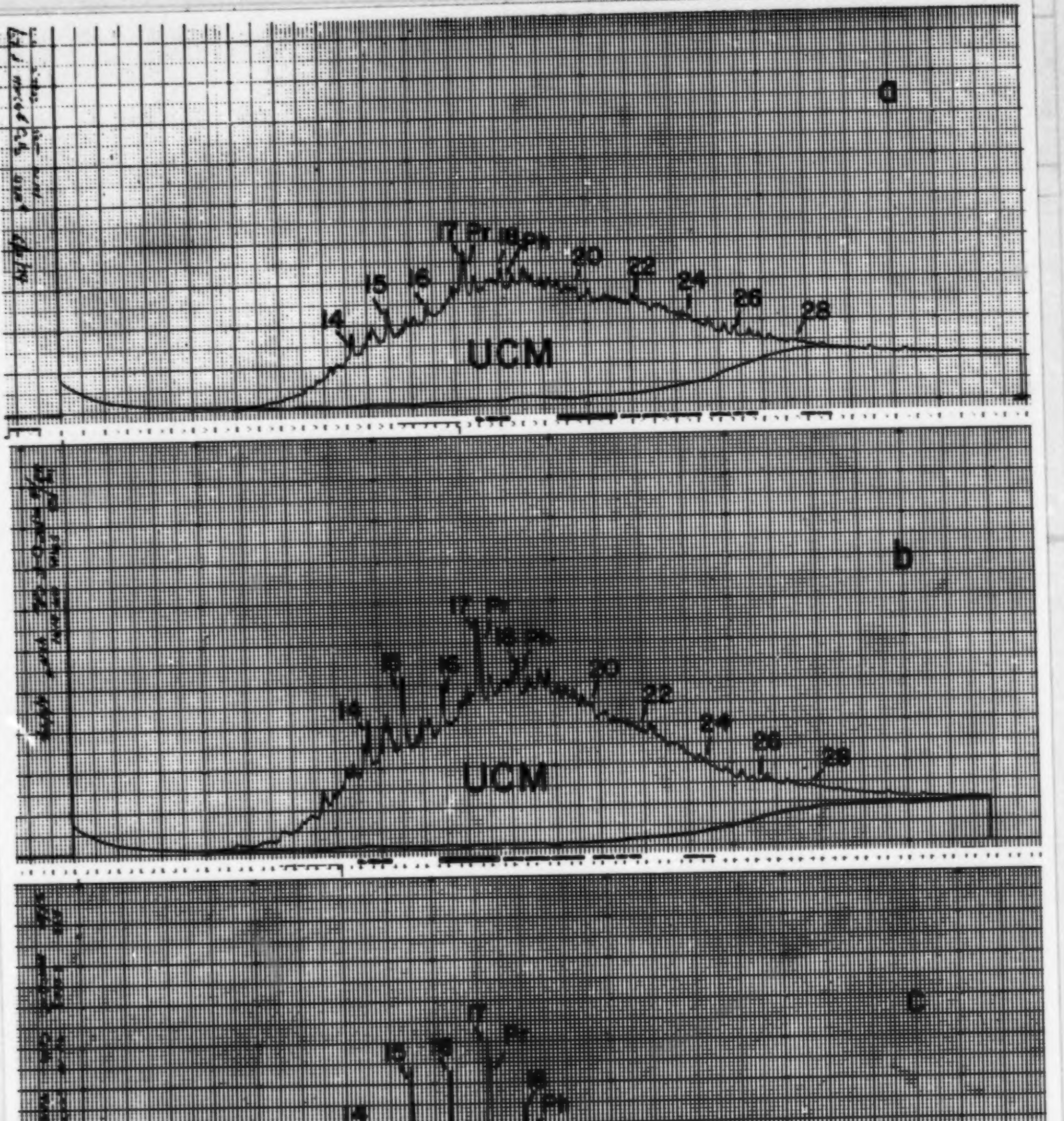

Figure 9. Gas chromatograms of alkanes and cycloalkanes from Narragansett Bay Station C clams and clams spiked w/No. 2 fuel oil. a - unspiked M. mercenaria; b - M. mercenaria spiked w/1 ppm No. 2 fuel oil; c - M. mercenaria spiked w/10 ppm fuel oil.

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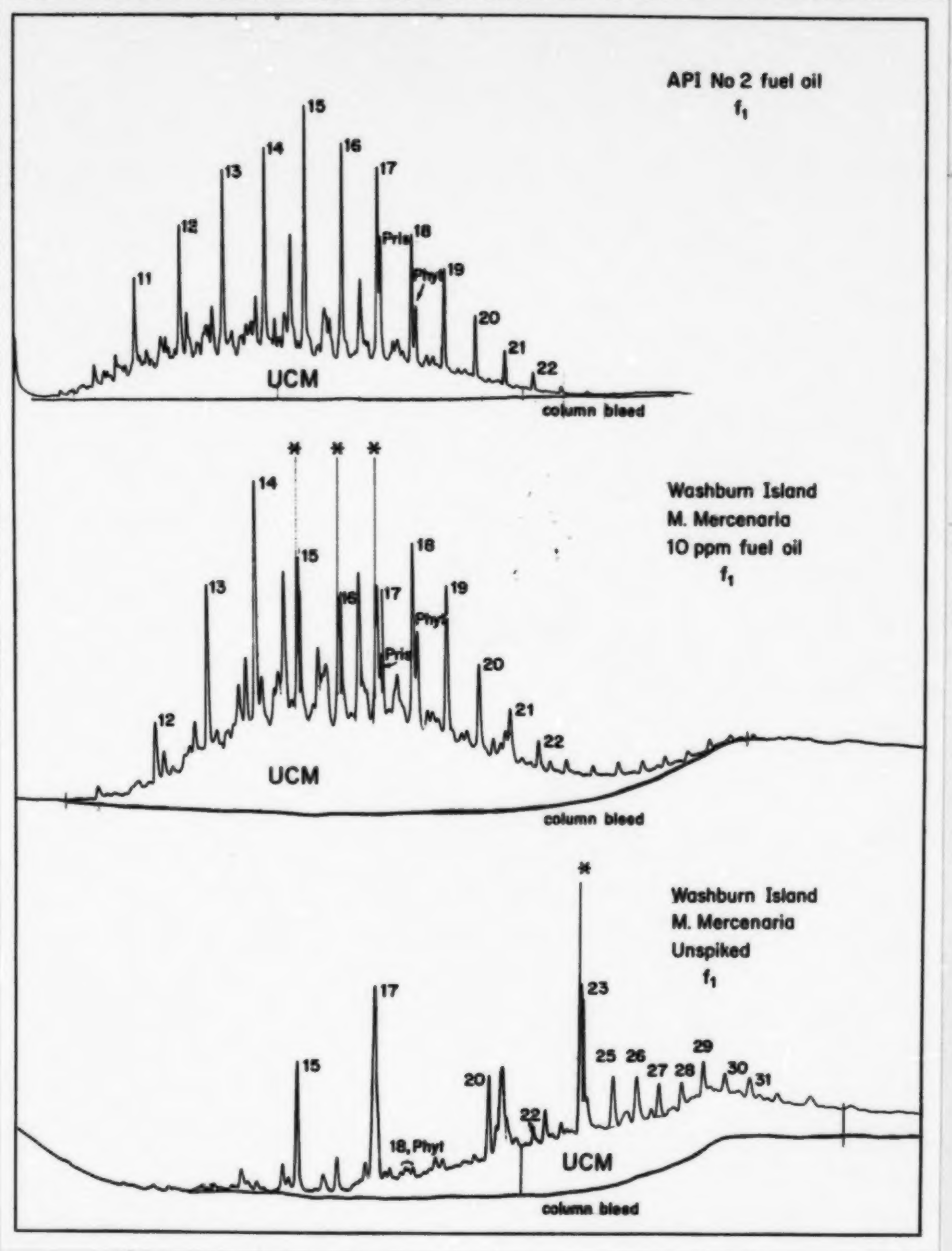

Figure 10. Gas chromatograms of alkanes and cycloalkanes isolated from Washburn's Island clams spiked w/API No. 2 fuel oil.

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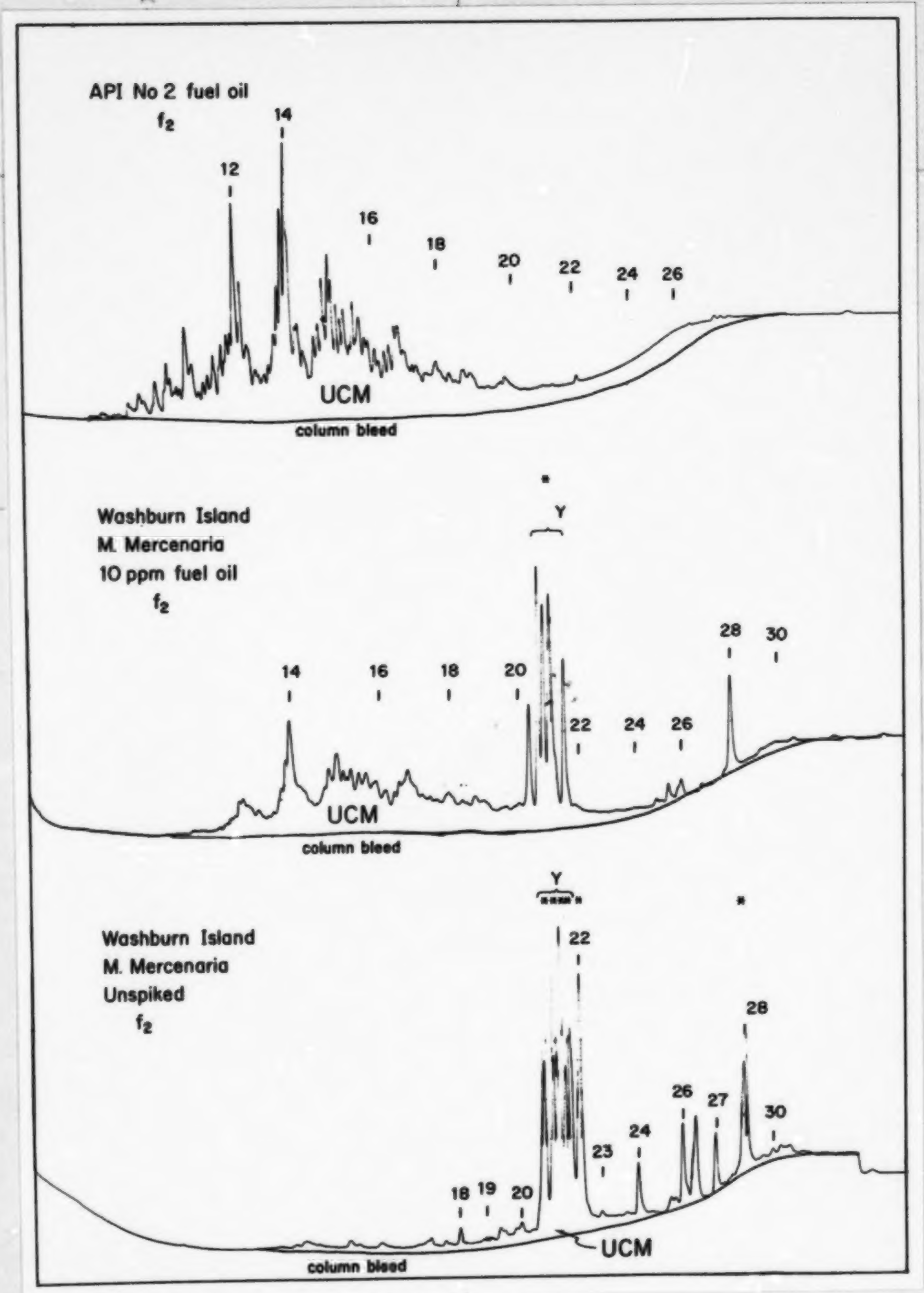

Figure 11. Gas chromatograms of aromatic hydrocarbon fraction of Washburn's Island clams and clams spiked w/No. 2 fuel oil.

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TABLE 3. RESULTS OF ANALYSES OF CLAM HOMOGENATE SPIKED WITH 10 µg API NO. 2 FUEL OIL/g WET WT.

METHOD	LOCATION		µg/g WET WT.				TOTAL
			f_1		f_2		
Virtis Homogenization	Station C						
	Spiked		11.25		4.50		
			11.35		5.20		
		Ave.	11.30		4.85		
	Unspiked		6.59 ± 1.23		2.07 ± 1.05		
	Spiked-Unspiked		4.71	+	2.78	=	7.49
Digestion	Station C						
	Spiked		11.04		5.13		
			13.82		6.26		
		Ave.	12.43		5.69		
	Unspiked		6.57 ± 0.84		3.00 ± 0.41		
	Spiked-Unspiked		5.86	+	2.69	=	8.55
Digestion	Washburn's Island						
	Spiked		6.89		4.72		
	3 Analyses		5.67		4.68		
			5.50		4.61		
		Ave.	6.02		4.67		
	Unspiked		1.88		1.83		
	4 Analyses		1.69		1.95		
			0.95		1.88		
			1.35		2.15		
		Ave.	1.46		1.95		
	Spiked-Unspiked		4.56	+	2.72	=	7.28

chromatography cut of the API No. 2 fuel oil, and the f_1 fraction from extraction of spiked clam homogenate to illustrate this point. The results are given in Table 4 and demonstrate that some alterations to the gas chromatographic tagging parameters occur during column chromatography, probably as a result of the removal of aromatic hydrocarbons from the saturated hydrocarbons. It is also clear from the results that interference from natural biogenic hydrocarbons already in the sample can cause the passive tagging parameters to change. This suggests that gas chromatographic passive tagging schemes which rely on ratios of normal and branched alkanes may be useless when applied in an attempt to correlate low concentrations of petroleum pollutants in organisms with the source of contamination.

A comparison of determinations of total hydrocarbons in column chromatography fractions f_1 and f_2 determined by gravimetric analysis on the Cahn electrobalance and gas chromatographic analyses using external standard calibration of the flame ionization detector is given in Table 5. In general the agreement is quite good, although a few values are definitely out of line. This may be due to differences in the hydrocarbons detected in the molecular weight range of the GC analyses, n-C_{14} to n-C_{31}, and the total hydrocarbons of molecular weight greater than n-C_{14} by the gravimetric Cahn electrobalance determination.

The precision attainabale for GC analyses for petroleum hydrocarbons in a spiked sample is illustrated in Table 6 and Table 7 and is illustrated by comparing the gas chromatograms of duplicate subsamples in Figures 12 and 13.

We also analyzed the samples given in 13 by gas chromatography-mass spectrometry. We were not at all satisfied with the results we obtained using a SCOT OV-101 column. The results obtained using a packed DEXSIL 300 column did not provide us with the desired resolution. However, we did obtain mass spectral data confirming the presence of several fuel oil aromatic hydrocarbons in the spiked clams. This is illustrated in Figures 14-17 and illustrates the poor quality data obtained using packed gas chromatography-mass spectrometry, compared to glass capillary gas chromatography-mass spectrometry which we will discuss in section 10 of this report.

It is sufficient to state here that during the period of our investigations gas chromatography technology improved markedly as we pointed out in the recommendations section.

UV-Fluorescence Analyses

Washburn's Island is the least contaminated of the locations we sampled. Figure 18 shows fluorescence emission spectra of a fraction 2 at about 1 ppm in MCH solution. The fluorescence emission is weak, yet significant since this determines the lower detection and identification limit for oils fluorescing in the 300 to 375 nm wavelength region. This emission suggests the presence of low levels (estimated at 10 ppb from comparison to spectra of authentic standards) of two ring aromatics in the

TABLE 4. RATIOS OF n-C_{17}, PRISTANE, n-C_{18}, AND PHYTANE IN API NO. 2 FUEL OIL SPIKED CLAMS.

API NO. 2 FUEL OIL		n-C_{17}/PRIS.	n-C_{18}/PHYT.	PRIS./PHYT.	n-C_{17}/n-C_{18}
Four analyses		1.62	2.03	2.15	1.72
API No. 2 fuel oil fraction 1 (three analyses)		1.56	2.19	2.07	1.47
Station C clam homogenate spiked with No. 2 fuel oil f_1		2.21	1.90	1.81	2.10
Washburn's Island clam homogenate spiked with No. 2 fuel oil f_1	a.	1.86	2.10	2.12	1.86
	b.	1.59	1.91	2.43	2.02
Average of a. & b.		1.72	2.00	2.28	1.94

TABLE 5. COMPARISON OF TOTAL HYDROCARBON DETERMINATIONS BY GRAVIMETRY AND GAS CHROMATOGRAPHY.

SAMPLE CODE[a]	GRAVIMETRY µg/g WET WEIGHT	GAS CHROMATOGRAPHY µg/g WET WEIGHT
MMCD-A f_1	7.82	6.52
MMCD-A f_2	3.29	3.652
MMCD-C f_1	11.04	7.22
MMCD-E f_1	6.34	2.49
MMCD-F f_1	7.89	6.24
MMCS-D f_1	8.17	5.85
MMCV-E f_2	11.42	8.08
MMCV-H f_1	7.95	8.67
MMCV-H f_2	3.23	2.97
MMED-A f_1	57.48	58.5
MMWD-A f_1	0.51	1.08
MMWD-C f_2	1.83	.33
MMWD-C f_2	1.83	.54
MMWD-D f_1	1.69	.90
MMWD-D f_2	1.95	1.15
MMWD-E f_1	6.89	4.76
MMWD-E f_2	4.72	2.63
MMWD-F f_2	4.68	3.19
MMWD-K f_1	5.3	7.12
MMWD-L f_1	13.8	7.57

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[a] MM - *Mercenaria mercenaria*; CD - Station C Digestion Extraction; V - Vertis Extraction; S - Soxhlet Extraction; A f_1 - Subsample A fraction 1 (see text).

TABLE 6. COMPARISON OF DUPLICATE GAS CHROMATOGRAPHIC ANALYSES OF ALKANES AND CYCLOALKANES IN NO. 2 FUEL OIL SPIKED WASHBURN'S ISLAND CLAMS.

	μg/g WET WEIGHT	
	MMWD-L	MMWD-K
n-C_{13}	.095	.121
x[a]	.087	.128
n-C_{14}	.185	.197
y	.169	.164
n-C_{15}	.291	.243
n-C_{16}	.228	.180
z	.070	.069
n-C_{17}	.256	.216
Pristane	.138	.136
n-C_{18}	.137	.107
Phytane	.065	.056
n-C_{19}	.106	.080
n-C_{20}	.072	.054
w	.028	ND[b]
n-C_{21}	.045	.066
n-C_{22}	.028	ND
UCM	5.57	

---

[a] w, x, y, z are compounds of unknown specific structure. x, y, z are probably isoprenoid alkanes based on GC-MS and GC retention time data.

[b] ND - Not Determined.

TABLE 7. COMPARISON OF DUPLICATE GAS CHROMATOGRAPHIC ANALYSES OF FRACTION 2 AROMATICS AND ALKENES IN NO. 2 FUEL OIL SPIKED AND UNSPIKED WASHBURN'S ISLAND CLAMS.

	µg/g WET WEIGHT			
PEAK NO.	MMWD-E	MMWD-F	MMWD-D	MMWD-C
13 A	.026	.023	ND[a]	ND
13 B	.059	.054	ND	ND
14	.079	.080	ND	ND
20 A	.048	.055	.082	.039
20 B	.090	.098	.139	.087
20 C	.093	.102	.128	.070
21	.234	.228	.340	.162
21 A	.024	.023		.057
21 B	.058	.068	.104	ND
28	.066	.072	ND	ND

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[a] ND - Not Determined - 13 A, 13 B, 14 not detected.

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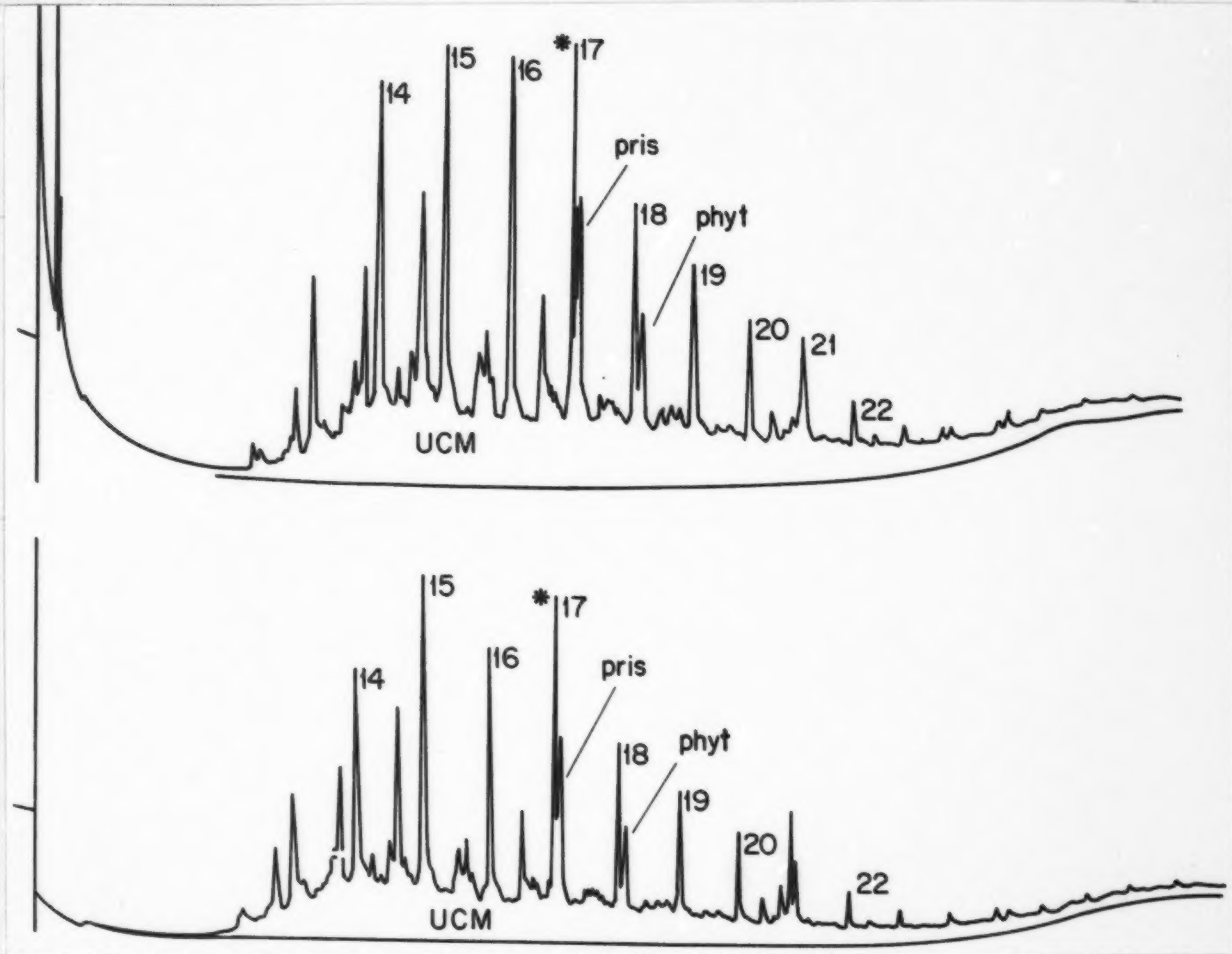

Figure 12. Comparison of gas chromatograms of alkanes and cycloalkanes from duplicates of Washburn's Island clams spiked w/No. 2 fuel oil.

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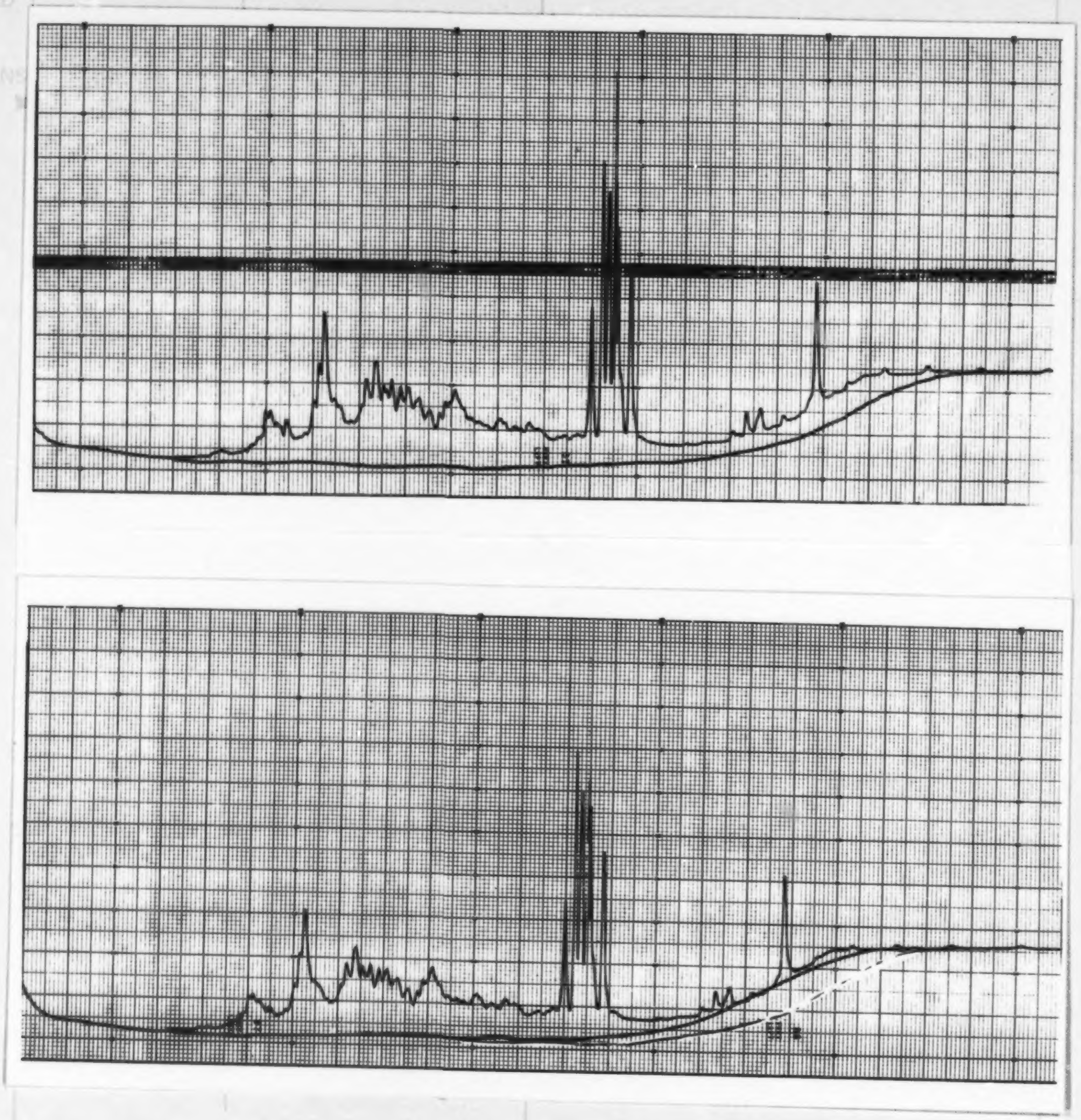

Figure 13. Comparison of gas chromatograms of aromatic hydrocarbons from duplicates of Washburn's Island clams spiked w/No. 2 fuel oil.

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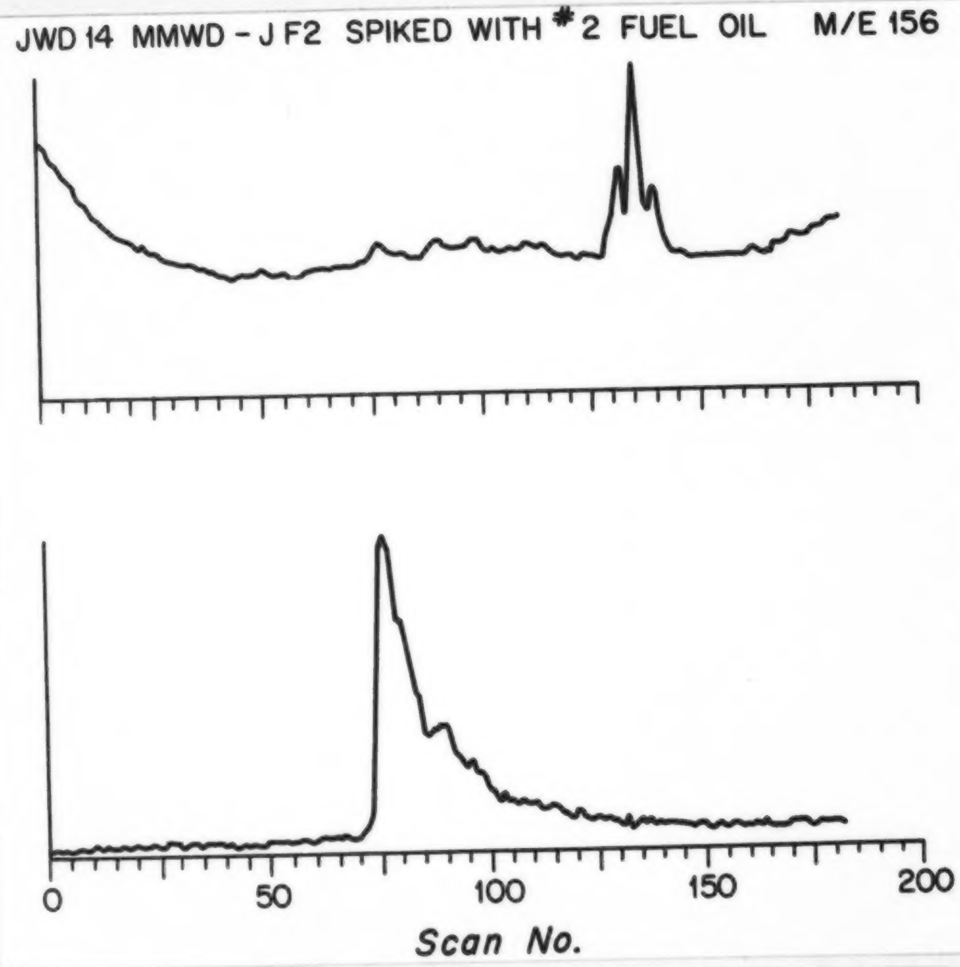

Figure 14. Computer reconstructed plot of total ion and m/e 156 ion intensity for gas chromatography - mass spectrometry analysis of aromatic hydrocarbons in API No. 2 fuel oil spiked Washburn's Island clams.

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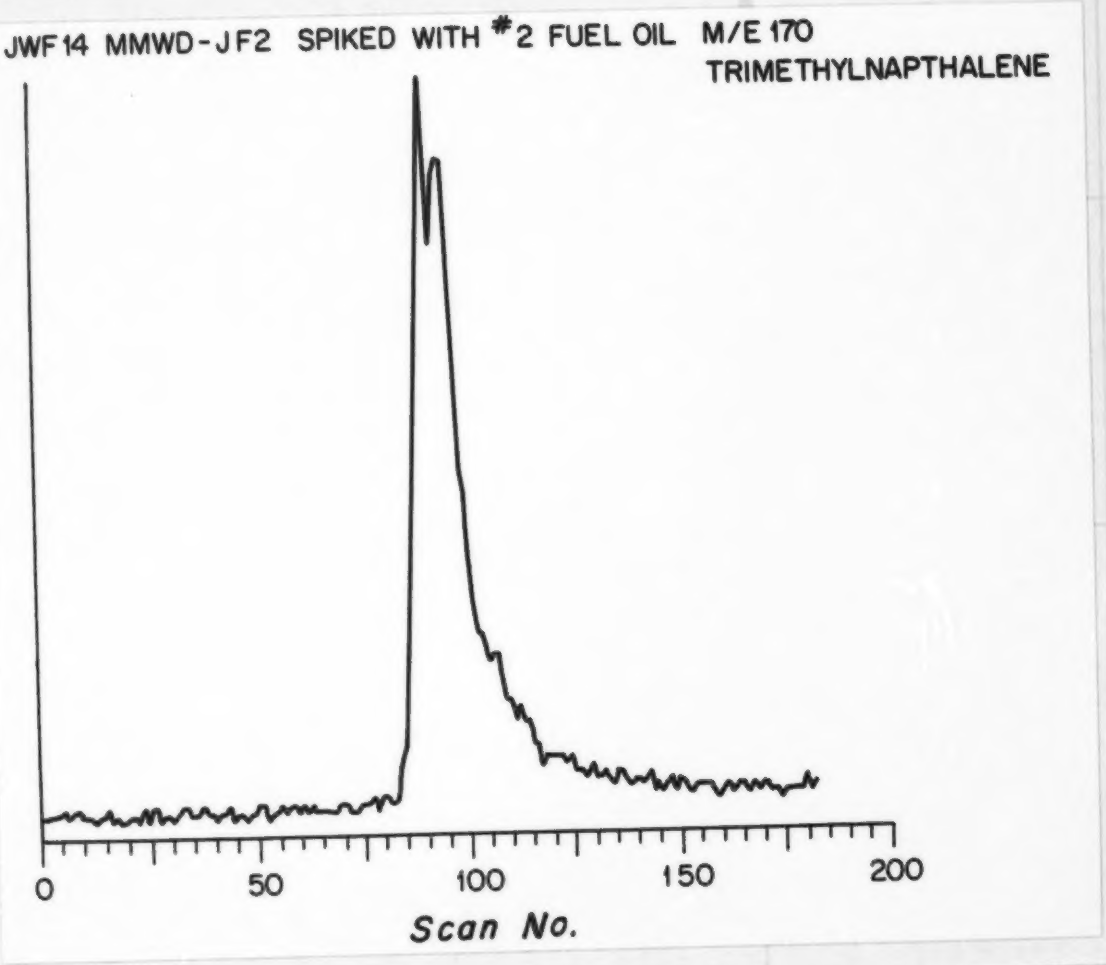

Figure 15. Computer reconstructed plot of m/e 170 ion intensity for sample described in Figure 14.

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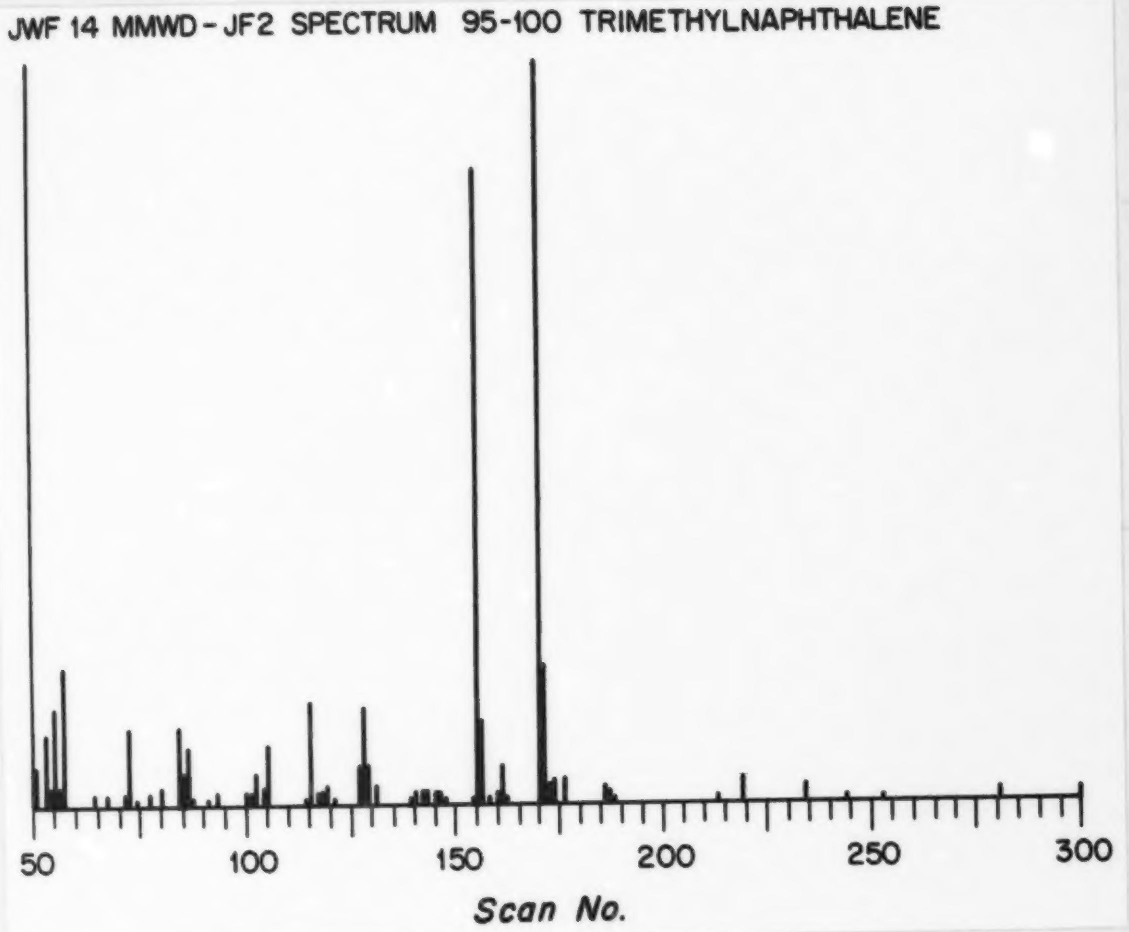

Figure 16. Mass spectrum of trimethyl naphthalene in API No. 2 fuel oil spiked clams.

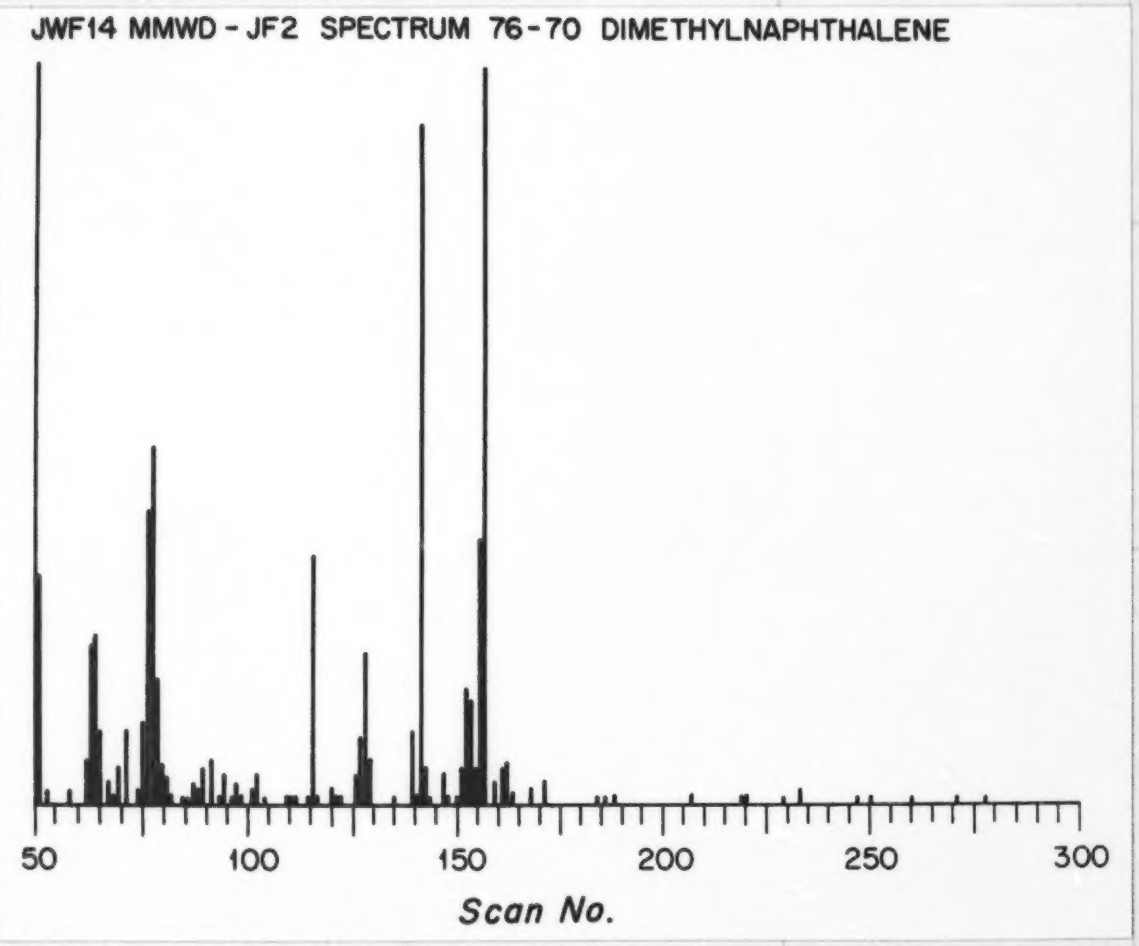

Figure 17. Mass spectrum of dimethyl naphthalene in API No. 2 fuel oil spiked clams.

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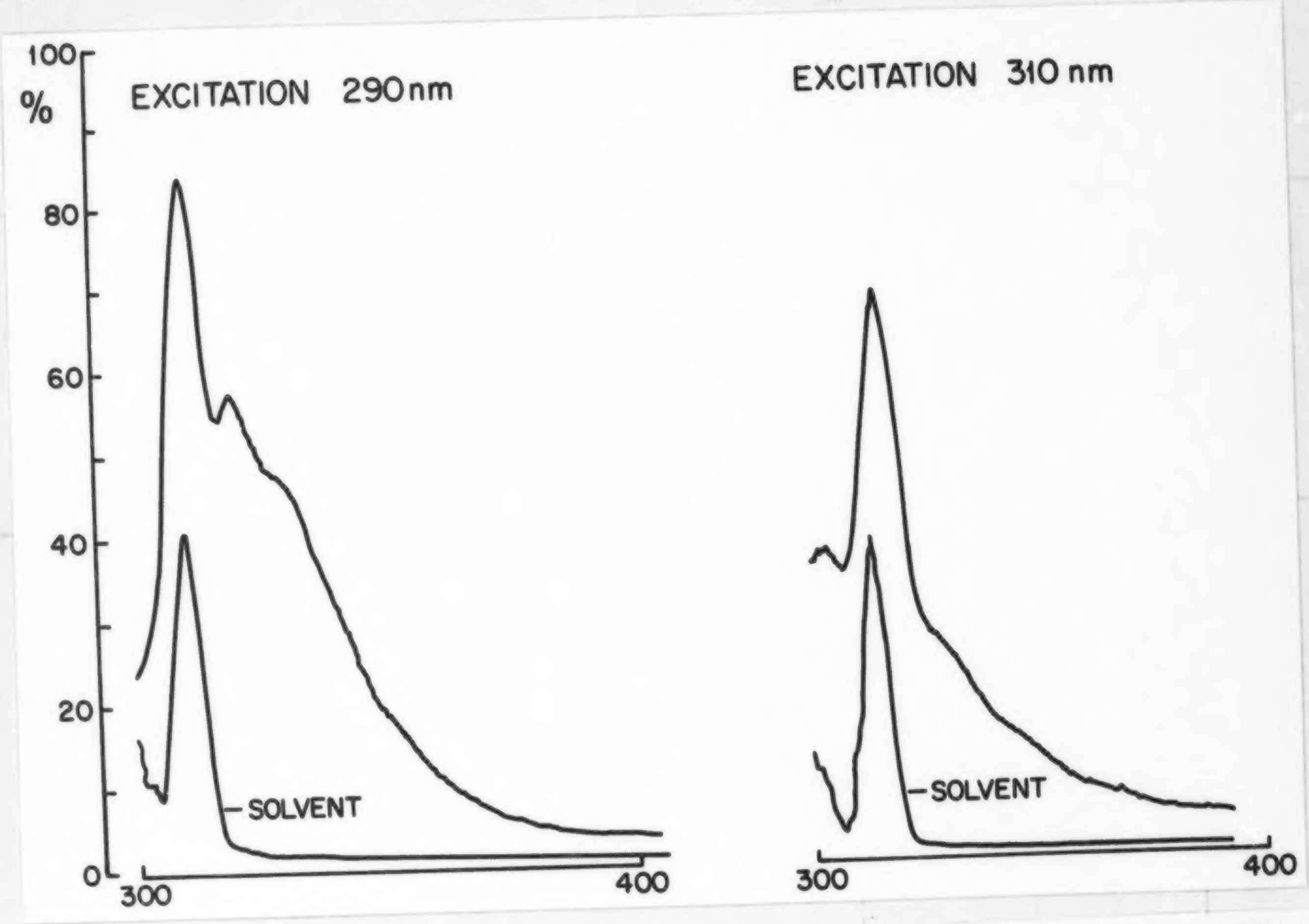

Figure 18. UVF spectra of aromatic fraction of Washburn's Island clams (slit 6x6; sensitivity 10).

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clam tissue.

Fluorescence emission in fraction 3 (Figure 19) is extremely weak. A concentration of about 20 ppm in solution is necessary to produce a measurable emission spectrum. The fraction 3 emission envelopes are somewhat broader than those from fraction 2 indicating the presence of higher molecular weight aromatics. Since the emission maxima occur near 350 nm, it is probably that most of these compounds contain no more than three rings.

Clams from station C were found to be moderately contaminated as previously discussed. Gas chromatography of fractions 1 and 2 showed a complex mixture of hydrocarbons largely unresolved in medium resolution columns.

When excited at 290 nm (Figure 20) a typical fraction 2 produces an emission envelope about 100 nm wide with an emission maximum centered at 329 nm. The intensity and position of the fluorescence emission suggests a significantly greater concentration (~ 10^2 ppb total) of two-ringed aromatics than in the Washburn's Island clam tissue. Similar to the Washburn's Island extract the station C fraction 3 produces a broad emission envelope at relatively high concentration in solution (Figure 20). The positions of the emission maxima gain suggest that three ring aromatics predominate in this fraction.

Fractions 2 and 3 from station E clams (Figure 21) exhibit very broad (~ 170 nm in width) fluorescent emission envelopes indicative of a very complex mixture of polynuclear aromatics in the clam tissue. This is consistent with the gas chromatographic results obtained on these extracts. The narrow emission bands at 365, 403 and 428 nm in both of the fraction 2 spectra result from a single compound present in high concentration in these clams. This emission pattern is also evident to a lesser extent in fraction 3 spectra of Washburn's Island and station C clam extracts.

The emission spectra of API No. 2 fuel oil fraction 2 are shown in Figure 22. The emission maximum in each spectrum occurs at 327 nm. The emission envelope excited at 290 nm is quite narrow indicating that two ring aromatics are the probable source of most of the fluorescence. This is consistent with the analysis of the oil which gives the diaromatic content as 8% (139), and it is consistent with the expected chromatographic mobility for this class of hydrocarbons.

The emission spectra of the Washburn's Island clam fraction 2 spiked at 10 ppm (μg/g wet wt.) API No. 2 fuel oil are nearly identical with those of the fuel oil fraction (Figure 23). Intensity (Table 8) of some shoulders and peaks are very comparable and provide verification that the envelopes are of the same shape. Figure 23 shows Washburn's Island spiked and unspiked extracts and No. 2 fuel oil fluorescence emission spectra excited at 290 nm and run at the same instrumental settings. Clearly the emission from the unspiked sample is not significant compared to that from the spiked sample at comparable concentrations in solution. The emission,

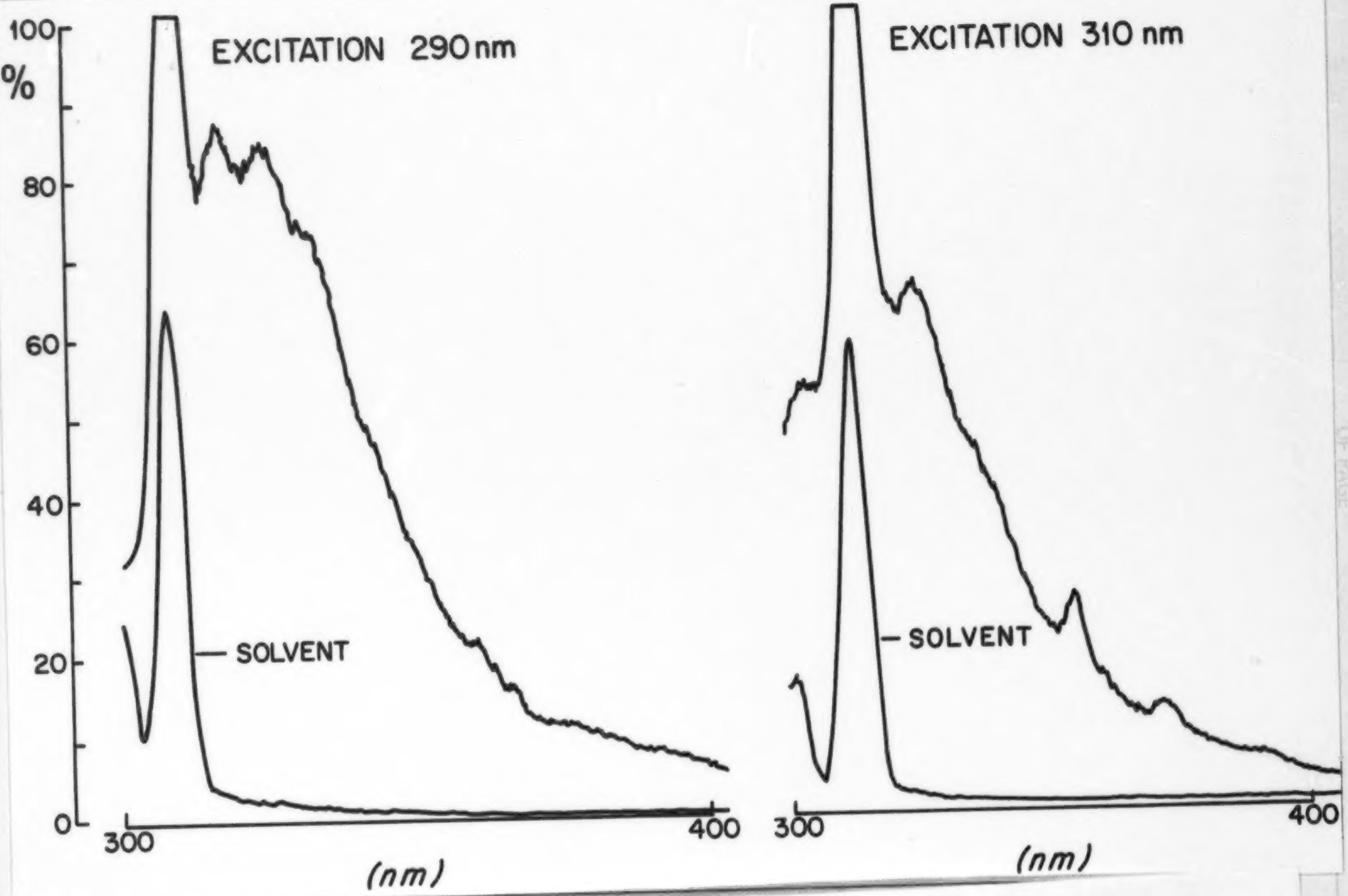

Figure 19. UV fluorescence spectra of aromatic fraction f_3 of Washburn's Island clams (slit 6x4; sensitivity 30).

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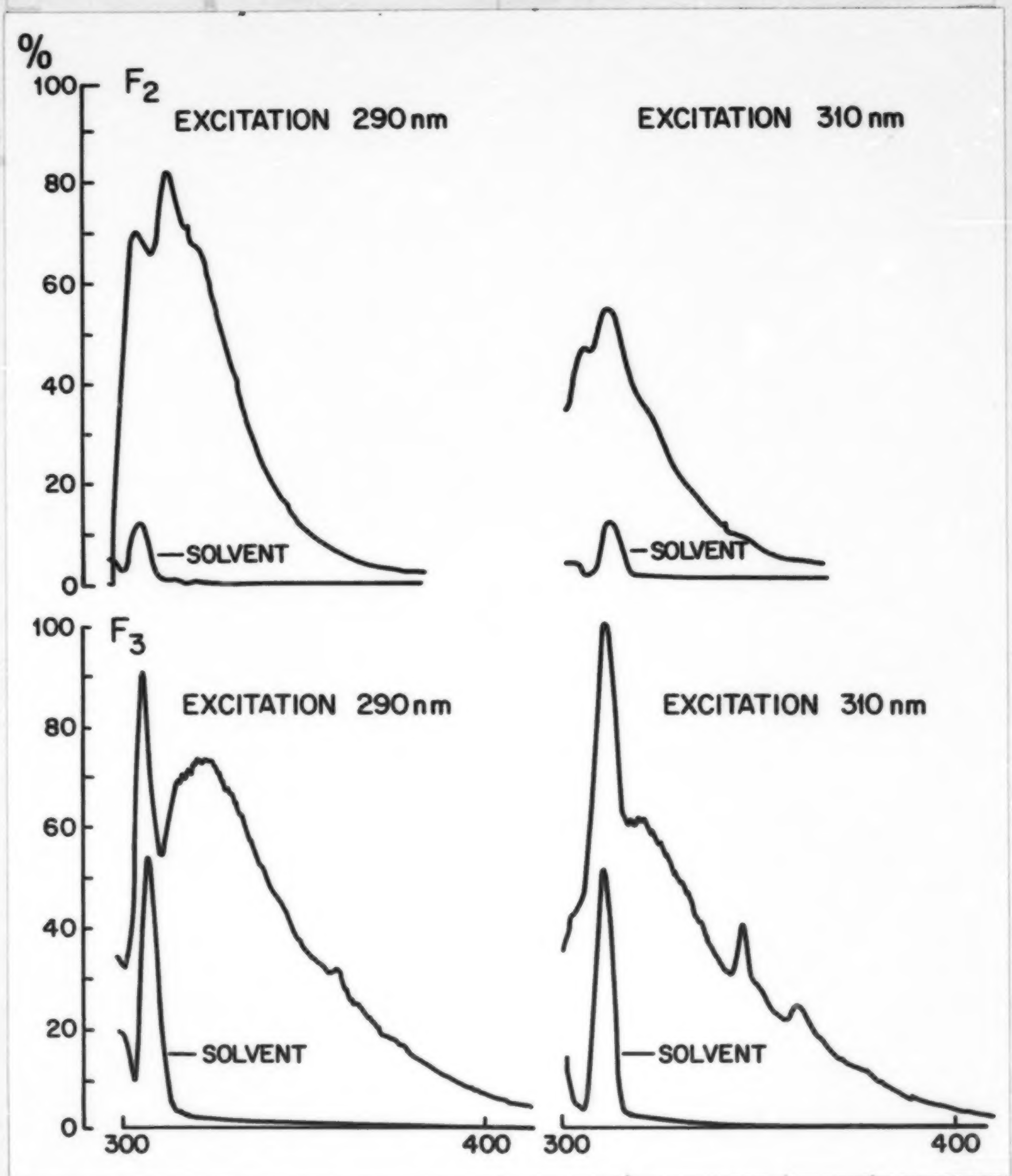

Figure 20. U.V. Fluorescence spectra of aromatic fractions (f_2 and f_3) Station C clams.

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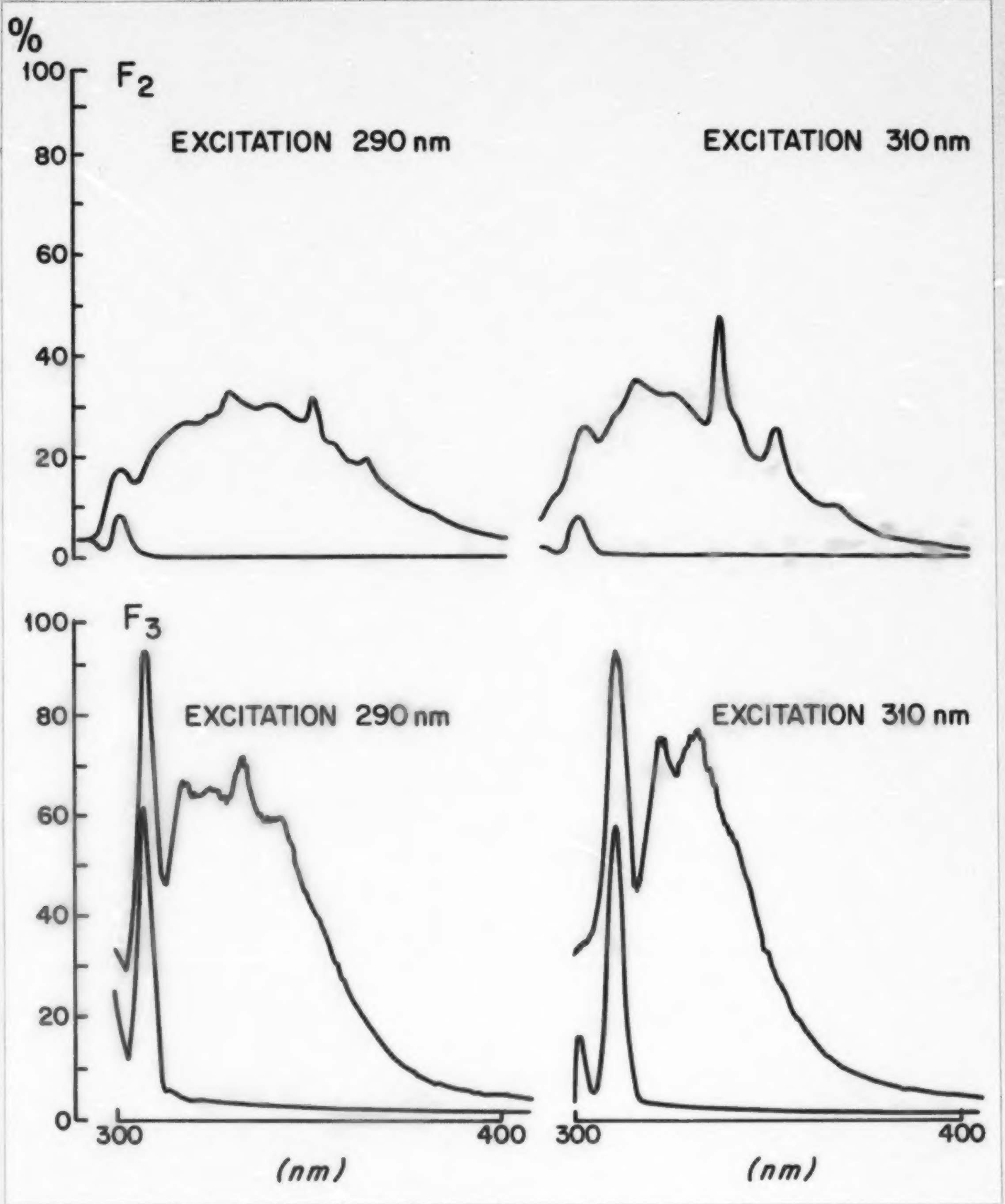

Figure 21. UV fluorescence spectra of aromatic fractions f_2 and f_3 Station E clams.

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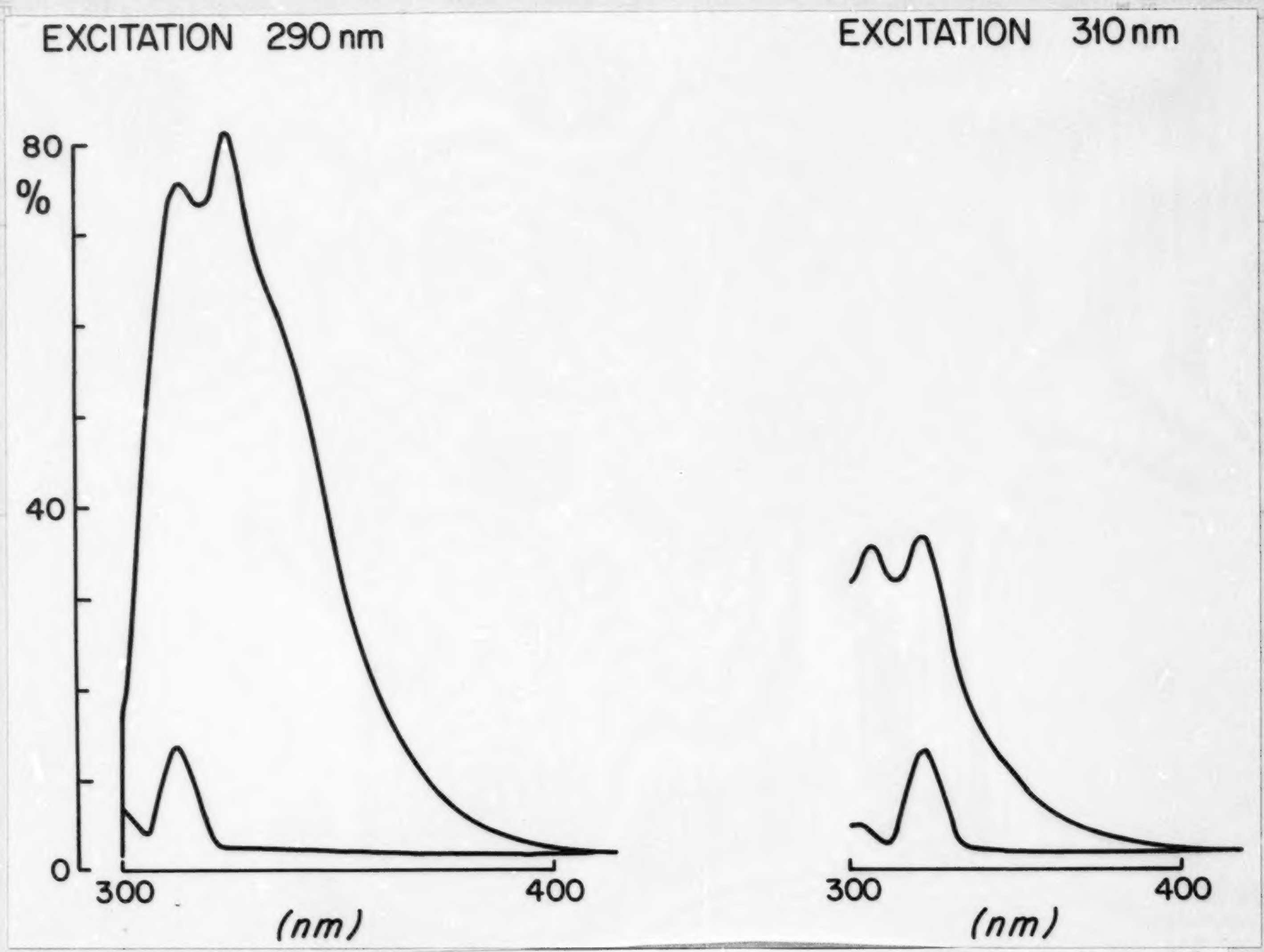

Figure 22. UV fluorescence spectra of API No. 2 fuel oil aromatic fraction f_2 (slits 6+6; sensitivity 3).

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TABLE 8. INTENSITY RATIOS FROM EMISSION SPECTRA.

RATIO	WASHBURN'S ISLAND SPIKED f_2	STATION C SPIKED f_2	STATION C UNSPIKED	NO. 2 FUEL OIL f_2
a I_{315}/I_{327} ex_{290} ex_{290}	0.82	0.68	0.70	0.79
b I_{327}/I_{327} ex_{310} ex_{290}	0.45	0.39	0.52	0.40
c b/a	0.55	0.57	0.74	0.51

therefore, from the spiked sample arises almost entirely from hydrocarbons in the No. 2 fuel oil spike.

Thus, a 10 ppm spike of this particular No. 2 fuel oil can be easily detected and identified in the presence of other hydrocarbons extracted from the rather clean Washburn's Island clams. It is not clear, however, that one could distinguish this No. 2 fuel oil from another No. 2 fuel oil with this method. It is also apparent that a 1 ppm spike of this fuel oil would be difficult to distinguish from the background fluorescent moiety in the clam tissue. More spiking experiments must be performed before the exact limits of this method on clean clams can be determined.

Figure 23 contains fraction 2 emission spectra of unspiked station C clams, 10 ppm spiked station C clams and API No. 2 fuel oil, respectively. The similarity of the three emission spectra (ex. 290 nm) is clear in Figure 23. The first two intensity ratios in Table 8 do not distinguish the spiked from the unspiked samples. The third ratio does seem to provide an index, albeit not an extremely reliable one, for the identification of the oil in the presence of other fluorescence emitters.

Synchronous excitation of emission ($\Delta\lambda$ = 23 nm) (140, 141) provides another basis for distinguishing the fuel oil spike. Figure 24 shows the synchronous excitation of emission spectra for the same samples shown in the previous figures (background subtracted). Resolution of the doublet for spiked sample is intermediate between that for the fuel oil and the unspiked sample.

These two methods, ratio comparison and synchronous excitation of emission are useful for identification in this case because the fuel oil has more fluorescence emitters in a narrower molecular weight range than the unspiked station C sample. Nevertheless, the fluorescence emission spectra reflect a similar composition of aromatics in each. Several possible explanations for this similarity are suggested. (a) A significant spill of No. 2 fuel oil may have occurred just prior to our sampling, but this is ruled out by our gas chromatographic data as

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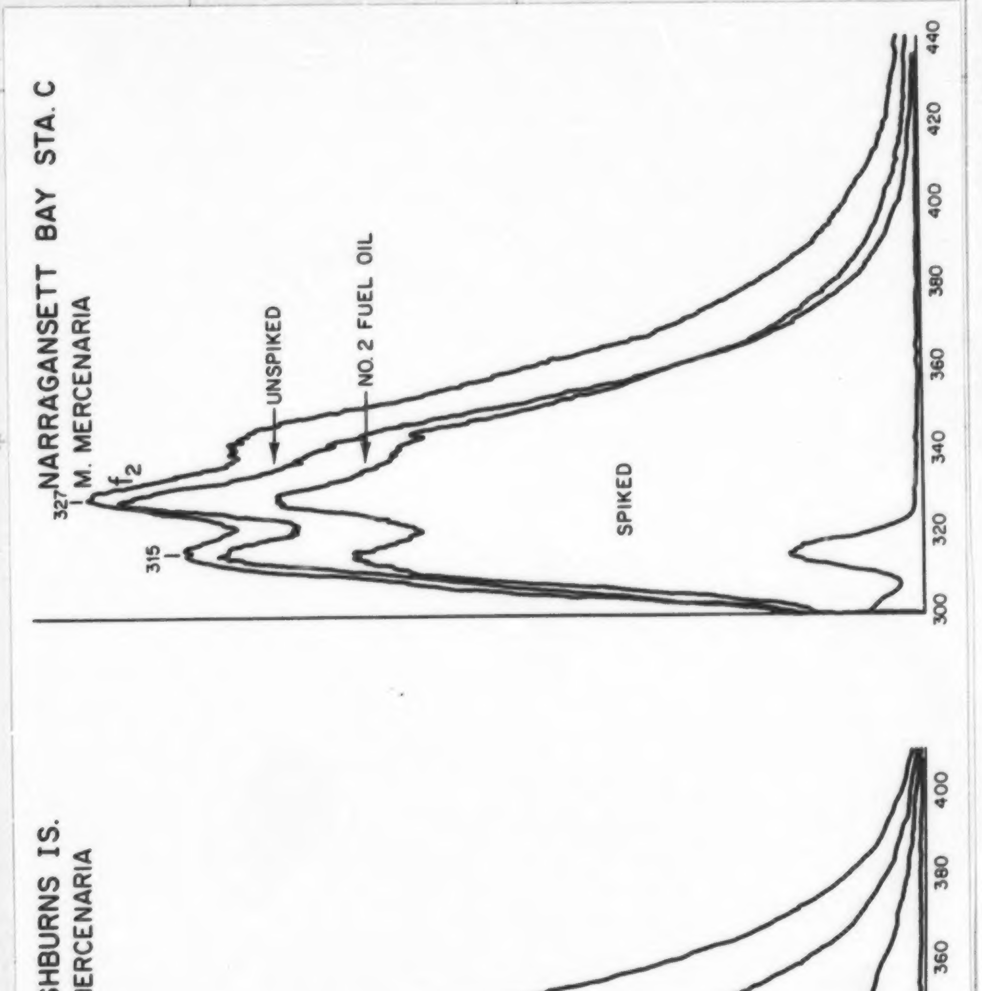

Figure 23. Comparison of UV fluorescence spectra of aromatic fraction f_2 of Station C and Washburn's Island clams and clams spiked w/10 ppm API No. 2 fuel oil.

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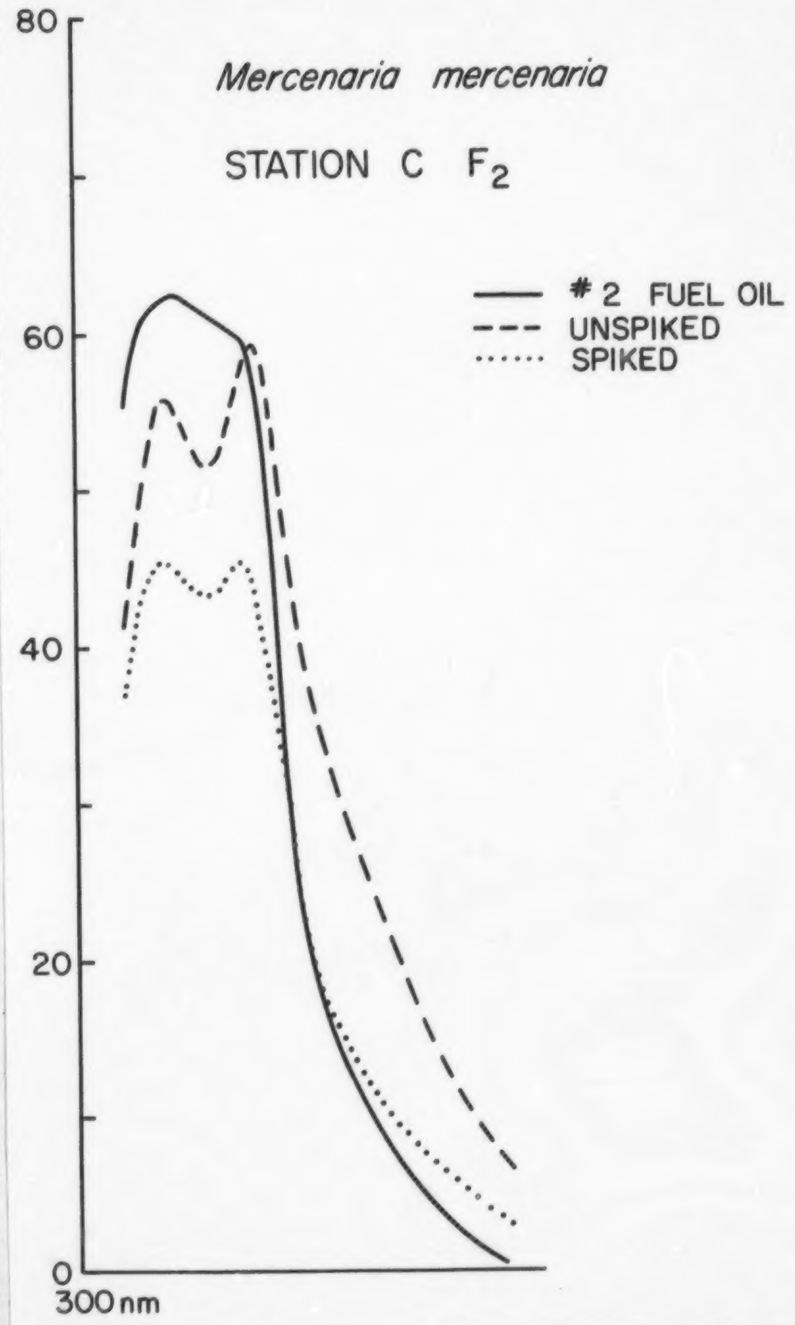

Figure 24. Synchronous excitation-emission UV fluorescence spectra for aromatic fraction f_2 of station C Clams, API No. 2 fuel oil, and clams spiked w/10 ppm No. 2 fuel oil.

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previously discussed. (b) A significant spill of No. 2 fuel may have occurred well before our sampling. This is not supported by the gas chromatograms either. However, the rates of depuration of one hydrocarbon class relative to another are not well understood so this remains a possibility. (c) Light aromatics from Bunker oils spilled over the years at the Quonsett Point Naval Base (~ two miles north) have been solubilized into the water, carried south by currents and concentrated by the clams. The fluorescence emission from the water soluble portion of a crude oil has been reported and it bears a resemblance to the fluorescence emission of API No. 2 fuel oil (142).

Analysis of clams from several moderately contaminated locations will be needed to determine whether station C is typical or only a special case. If the station C fluorescence emission spectra are typical, then fluorescence analysis will not be useful for the determination of recently spilled light oils in clams already moderately contaminated with hydrocarbons.

We have found that so long as extracts are kept near 0°C in the dark the fluorescence emission from the spiked hydrocarbons can be easily related to the concentration of the spike. This is, of course, dependent upon the analyst's knowing the exact nature of the spike and having some material available for calibration purposes. The analyst must also have an unspiked control sample to establish the level of background fluorescence.

The potential of low temperature fluorescence spectroscopy for distinguishing between bulk oil samples has been demonstrated (143). The few preliminary low temperature experiments we have run indicate that this potential may extend to identification of oil in organisms. Figure 26 shows 77°K emission spectra of an unspiked station C fraction 2. Figure 27 shows 77°K emission spectra of No. 2 fuel oil, fraction 2. The spectra excited at 290 nm are still not extremely different, the only major difference being better resolution of several shoulders in the clam extract spectrum. Excitation at 310 nm, however, produces spectra that are clearly different, and excitation of the clam extract at 340 nm produces a spectrum with fine structure like that observed in No. 6 fuel oils at low temperature (143). These differences, arising presumably from enhanced resolution at low temperature, may afford the analyst a better opportunity to distinguish and identify emissions of recently spilled oil.

It seems unlikely that fluorescence spectroscopy is suitable to be a primary tool in studies of hydrocarbons in organisms. Like ultraviolet adsorption spectroscopy, it is limited by the numbers and types of compounds which undergo electronic transitions in the UV-visible range. While most of the compounds that do emit when excited with ultraviolet radiation are probably of geochemical origin, molluscs from many locations may already contain enough of these to foil fluorescence analysis in an oil spill situation.

The most fundamental problem lies in the fact that fluorescence spectra (at 25°C) of multicomponent mixtures such as oils are, like UV spectra, relatively featureless. They provide the analyst, therefore, a few

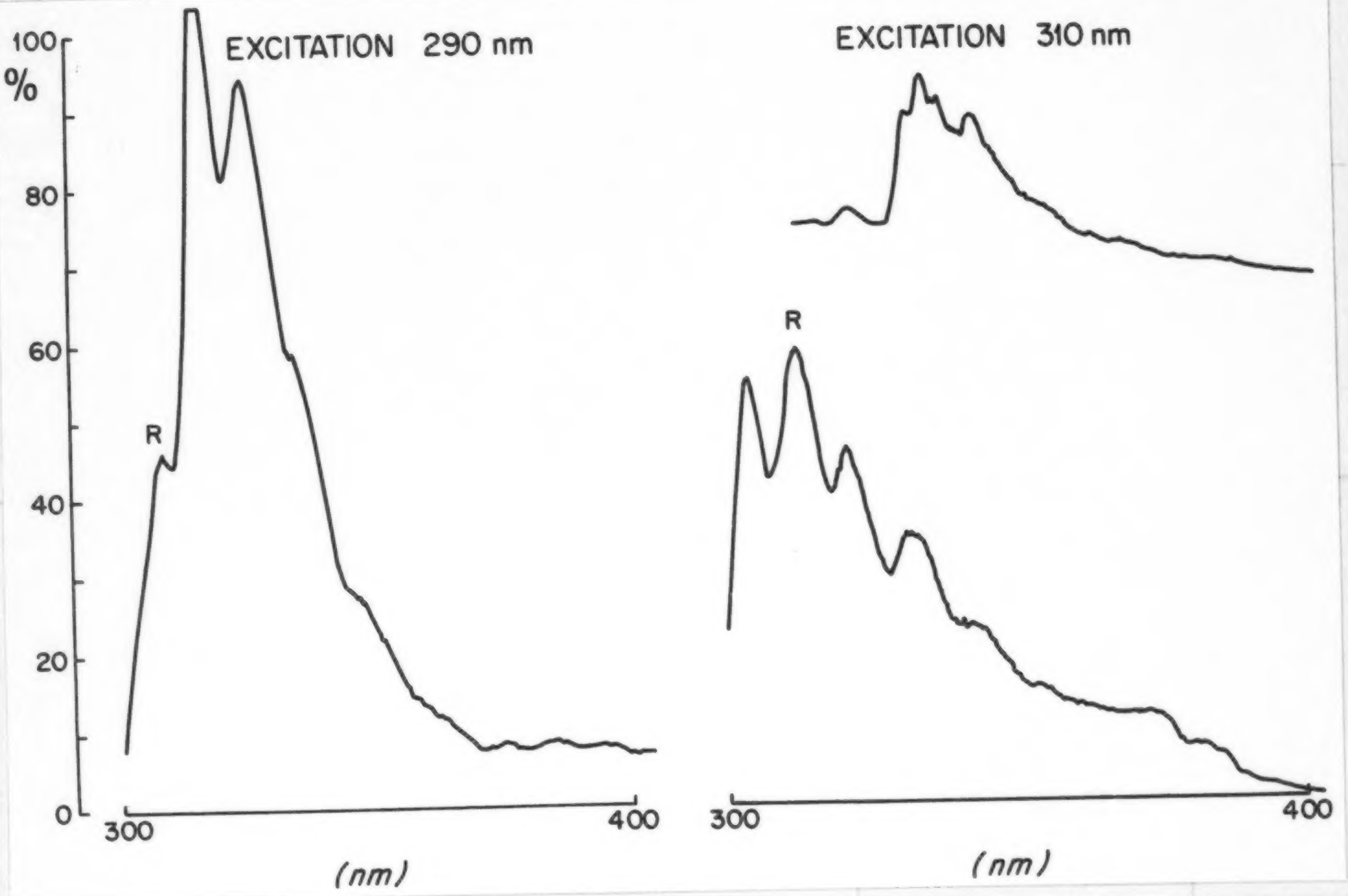

Figure 25. Low temperature 77^{0}K fluorescence spectra of aromatic fraction f_2 of station C clams. (slits 6+4; sensitivity 10).

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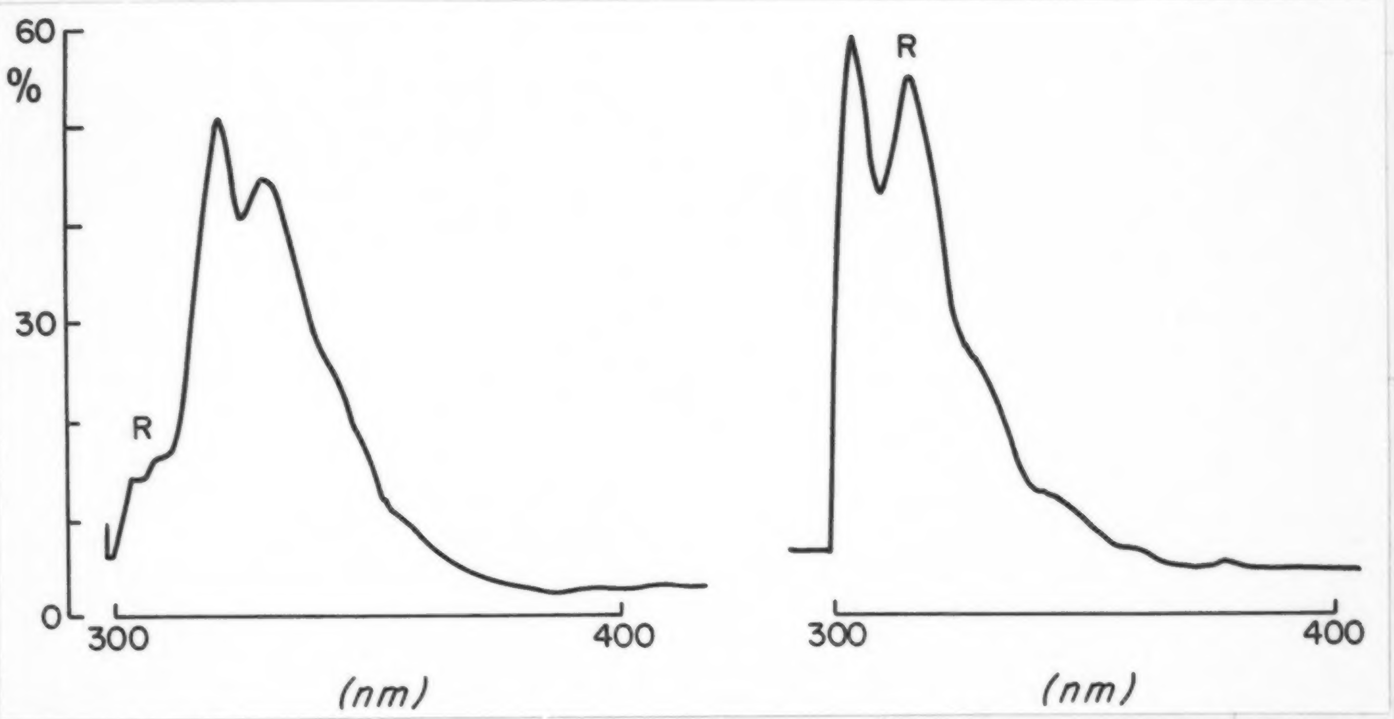

Figure 26. Low temperature $77^{0}K$ fluorescence spectra of aromatic fraction f_2 of API No. 2 fuel oil. (Slit 6+4; sensitivity 3).

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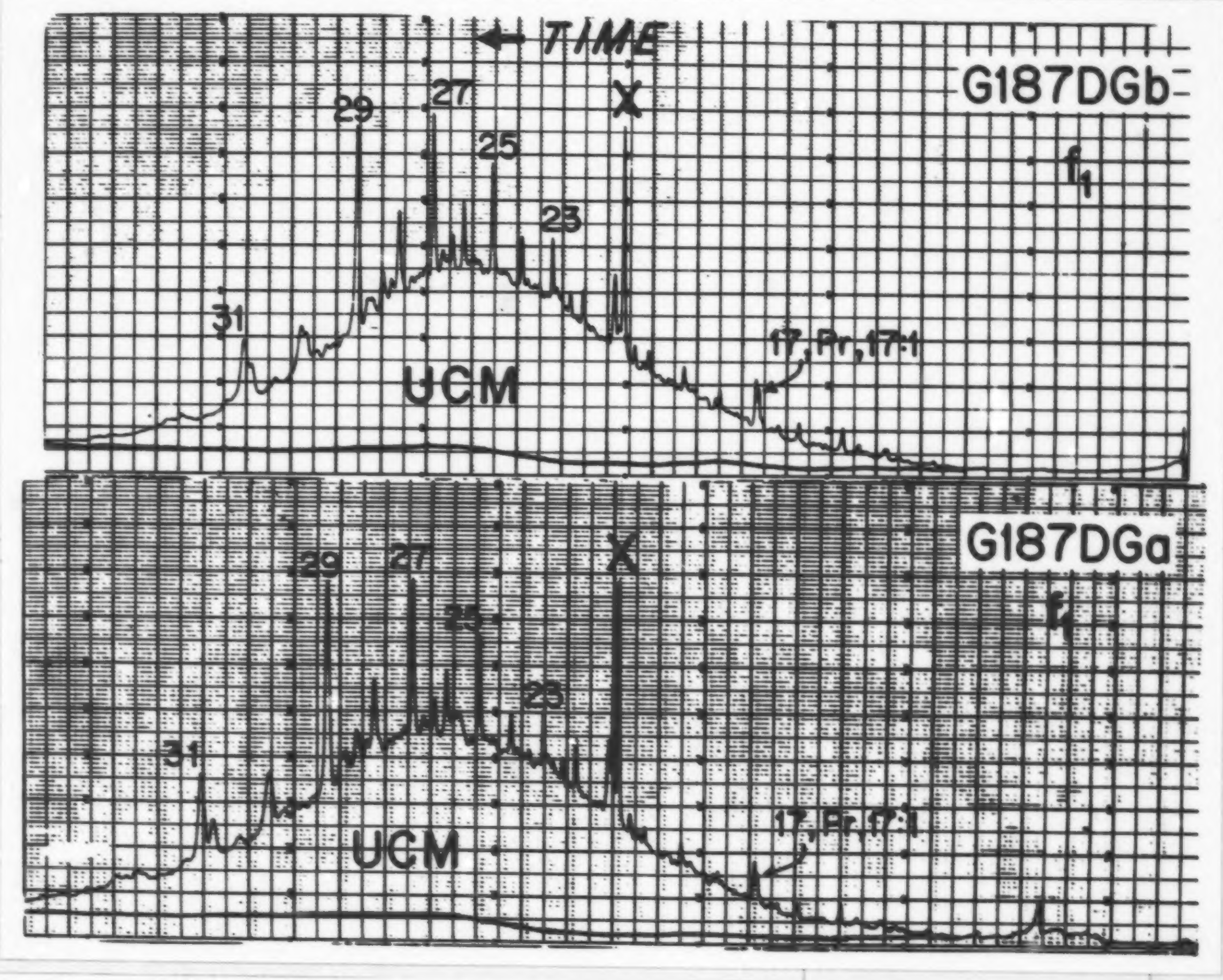

Figure 27. Gas chromatograms of alkanes, cycloalkanes, alkenes, and cycloalkenes from duplicate extractions of Hudson Channel surface sediments.

measurable parameters on which he can base comparisons. This situation may be remedied to some degree by obtaining spectra at 77°K. In the enhanced resolution and sensitivity of low temperature fluorescence lies, perhaps, its greatest potential for application to the problem of identification.

Quantification of oil in organisms is also possible, but again the selectivity of the fluorescence method constitutes its most serious drawback. While the flame ionization detector in a gas chromatograph produces approximately the same signal for all hydrocarbons, the photomultiplier in a fluorescence spectrophotometer "sees" emission from aromatics only. If organisms were to take up, release and/or metabolize all classes of hydrocarbons alike, the aromatic content would be a reliable index of the total hydrocarbon content. However, this is undoubtedly not the case.

Since it can provide rapid estimates of concentration and general composition of aromatics extracted from tissue, fluorescence spectroscopy remains a useful adjunct to other techniques such as GC and GC-MS. As a primary technique for characterizing petroleum residues in organisms, however, fluorescence spectroscopy is not the method of choice.

SECTION 6

COMPARISON OF SOME METHODS OF ANALYSIS FOR HYDROCARBONS IN SEDIMENTS

1. Introduction

We have examined the hydrocarbons obtained from three methods of extraction applied to a Narragansett Bay sediment. The sediment sample used was a large composite grab sample, taken from a location in West Passage that is relatively uncontaminated. During sampling, care was taken to avoid contact with all oils, lubricants, and plastics and the sample contacted only solvent rinsed stainless steel or glass.

In general, the extraction techniques used will separate a complex lipophilic mixture from the environmental sample. The preliminary extraction is followed by the separation of hydrocarbons from the other lipids and then the hydrocarbon mixture can be analyzed by a variety of techniques in an attempt to deduce the relative amounts of native and fossil hydrocarbons. A blank of the complete extraction procedure was regularly run along with samples to determine if interfering compounds are introduced during the analysis.

2. Experimental Methods

The Soxhlet extraction procedure with benzene:methanol has been used by several investigators (1-3) and has been modified to fit our circumstances. About 100 g wet weight, of spiked or unspiked sediment is weighed into a single thickness paper extraction thimble and Soxhlet extracted for 24 hours with 1:1 benzene:methanol. At the end of this period, the extract is transferred to a storage flask and the sample is further extracted with fresh solvent. The extraction is continued for a third day, again using fresh solvent after each twenty-four hour period.

The combined extract is transferred to a separatory funnel and shaken with acidic, saturated NaCl solution. The benzene phase is separated from the aqueous methanol phase. The latter is then extracted two more times with pentane. The combined pentane-benzene extract is back-washed with fresh saturated NaCl solution and dried over Na_2SO_4 overnight. All of the aqueous portion of the original extract is discarded.

The dried solution of total extractable lipid is evaporated at reduced pressure at or below room temperature. The sample is removed as soon as the pressure drops to ~ 5 Torr. Extracts are weighed directly on an analytical balance. The total extractable lipid is saponified under reflux for two hours with 0.5 N KOH in methanol:benzene 1:1 with 25% H_2O

added. This alkaline hydrolysis removes fatty acids, wax esters and triglycerides which are a part of the total lipid extract. It is important that 25% H_2O is added to this hydrolysis to prevent transesterification of esters which may be present. The hydrolyzed sample is cooled and extracted as above with salt solution and pentane. The pentane extract is evaporated as above and the residue weighed again.

An activated copper column is used to remove interfering elemental sulfur from the non-saponifiable lipid extract (60). The sample is charged to the column and eluted with three column volumes of pentane-benzene. The solvent is again evaporated and the sample weighed as above.

The sulfur-free, non-saponifiable lipid extract is further separated by chromatography through a column of (1:1) Al_2O_3 (alumina) packed over SiO_2 (silica). The Al_2O_3 and SiO_2 are activated overnight at 250°C and 120°C respectively and then both are deactivated with 5% water. The sample of sulfur-free, non-saponifiable lipids is charged to the column with a small volume of pentane. The ratio of column material to non-saponifiable lipids is 100:1 or greater. The following elution scheme is employed:

Fraction 1 f1 - 1 column volume of pentane
Fraction 2 f2 - 1 column volume of 10% benzene in pentane
1 column volume of 20% benzene in pentane
Fraction 3 f3 - 1 column volume of benzene

The three fractions are evaporated to dryness on a rotary evaporator at room temperature or slightly below with care taken to remove the sample vial as the pressure drops to ~ 5 Torr. The hydrocarbon fractions are then taken up in 50 to 200 μl of CS_2 and an aliquot of 5 to 10 μl is weighed on the Cahn Electrobalance. An aliquot of 1-5 μl is injected into the gas chromatographic column.

The second extraction technique is the direct alkaline hydrolysis of a sediment sample. Following the procedure of Farrington and Quinn (132), 50 g of the estuarine sediment is refluxed for three hours with benzene and 0.5 N methanolic KOH. After cooling, the solvent is filtered with suction and remaining sediment stirred with methanol and 1 N HCl. This mixture is also filtered and the residue is washed on the filter with methanol followed by pentane. The combined filtrate is extracted two times with pentane, the combined pentane extract is back-washed two times with distilled water and dried over Na_2SO_4. After evaporation of the solvent, the sample is weighed and then carried through the sulfur removal and column chromatography steps as described in the preceding discussion.

An extraction procedure using an acid wash prior to three days of Soxhlet extraction with benzene-methanol has also been tested. A sediment sample was washed two times with 1 N HCl and once with distilled H_2O, centrifuging between washings and saving the supernate. The sample is then weighed into an extraction thimble and extracted as described above. The sample is treated exactly as in the original Soxhlet extraction procedure except that the combined acid washings are added to the benzene-methanol extract during the re-extraction with pentane plus salt solution.

Each extraction technique used results in three separate fractions of hydrocarbons at the end of column chromatography. The data presented here are our interpretations of gravimetric analyses of the three fractions and gas chromatographic analysis of the pentane fraction from the column chromatography of non-saponifiable lipids. A packed OV-101 (5 ft., 1/8" stainless steel, 15% on 100/120 HP Chrom G, helium carrier gas at 15-20 ml/min) column and an OV-101 SCOT (Perkin-Elmer, 50 ft., helium carrier gas at 4-6 ml/min) column installed in Varian Model 1440 and 2740 gas chromatographs were used. The columns were routinely temperature programmed from 75°C to 275°C at 6°C/min, using a chart speed of 0.5 in/min.

The SCOT column is preferred because of its capability for higher resolution, but we have experienced some difficulty with the resolution of SCOT columns at the higher end of the molecular weight range that we encounter. Analysis of quantities of greater than 10 nanograms of each hydrocarbon produces sharp peaks with good resolution, but smaller amounts on the order of 1 nanogram or less result in poorer resolution and significant losses of the alkanes above n-C_{24}. Some of the probable causes of this effect such as split ratio, injector and/or detector temperature and flow rate have been investigated and we have concluded that these were not the cause. Some of the SCOT columns could be kept operating by periodic injection of a concentrated solution of crude oil. Evidently, the loss of resolution is due to an interaction between "hydrophobic" sites in the column and the sample material. Periodic coating of these sites with high molecular weight components of crude oil is usually sufficient to restore the operating capacity of the column.

A standard n-alkane mixture of known concentration is used to measure detector response per unit weight of alkane. The areas of peaks plus unresolved complex mixture are measured using planimetry and compared to the standard alkane mixture.

3. Results and Discussion

Comparison of Efficienies of Extraction Procedures - The result of comparison of three methods of extraction are given in Table 9. Based on our present data we conclude that there are no substantial differences in the efficiencies of the extraction procedures. Chemical treatment of the sediment by acid or base does not significantly increase the yield of hydrocarbons as compared to organic solvent extraction alone. This is in contrast to the extraction of fatty acids from sediments where KOH digestion or saponification extraction yielded a significantly greater amount of fatty acids (132). We do not have sufficient data to explain this difference and can only speculate that it may be the result of the differences in early diagenesis of the two classes of compounds.

The precision of the measurements of hydrocarbons in sediments is not as good as the precision for measurements of hydrocarbons in clams, *Mercenaria mercenaria*, sampled at the same locations as is indicated in Table 10. We think the poorer precision of the data for sediments reflects

TABLE 9. COMPARISON OF EFFICIENCIES OF SOME METHODS FOR EXTRACTING HYDROCARBONS FROM SURFACE SEDIMENTS[a].

EXTRACTION METHOD	NO. OF ANALYSES	μg/g DRY WEIGHT OF SEDIMENT[b] (AVERAGE ± STANDARD DEVIATION)			
		f_1	f_2	f_3	$\sum f_1 f_2 f_3$
Soxhlet w/Benzene: Methanol, 72 Hrs.	(5)	147 ± 54	25 ± 7	83 ± 30	265 ± 56
Digestion w/KOH:MeOH	(4)	136 ± 58	29 ± 6	45 ± 9	202 ± 54
HCl Prewash Followed[c] by Soxhlet w/Benzene: Methanol, 72 Hrs.	(2)	190 ± 55	43 ± 13	115 ± 20	347 ± 65
10 ppm Spike, No. 2[c] Fuel Oil Followed by Soxhlet Extraction	(2)	161 ± 19	40 ± 6	77 ± 9	279 ± 34

[a] Analyses of subsamples of a surface sediment sample. See text.

[b] f_1 – Alkanes, cycloalkanes, alkenes, cycloalkenes.

f_2 – Alkenes, cycloalkenes, 2 and 3 ring aromatics.

f_3 – 4–6 Ring aromatic hydrocarbons and traces of methylketones.

[c] Estimated standard deviation from two analyses.

TABLE 10. COMPARISON OF PRECISION OF SOXHLET AND DIGESTION EXTRACTION AND HYDROCARBON ANALYSES FROM CLAMS (*M. MERCENARIA*) AND SURFACE SEDIMENT SAMPLES FROM THE SAME LOCATION[a].

		RELATIVE STANDARD DEVIATION				
EXTRACTION METHOD	NO. OF ANALYSES	SEDIMENT		NO. OF ANALYSES	CLAM	
		f_1	f_2		f_1	f_2
Soxhlet w/Benzene:Methanol	(5)	37%	28%	(6)	11%	8%
Digestion w/KOH:MeOH	(4)	43%	22%	(6)	13%	14%

the greater dfficulty of obtaining a homogeneous mixture of the wet sandy silt sediment and reproducibly subsampling in comparison to the clam homogenate. The fact that the analysis of the hydrocarbons extracted from the clams yields a better precision indicates that the column chromatography and weighing of isolated hydrocarbons is not the source of the poorer precision for the sediment hydrocarbon data. This contention is also supported by analyses of hydrocarbons in marine lipids and marine tissues conducted as part of an intercalibration program (6). The poorer precision of the sediment hydrocarbon analyses may also be the result of differences in the molecular weight range of hydrocarbons being quantified in each case. Gas chromatographic analyses show that the heavier molecular weight hydrocarbons are more predominant in the sediment hydrocarbon mixture compared to the clam hydrocarbon mixture. It may be more difficult to obtain precise measurements of heavier molecular weight hydrocarbons due to difficulties associated with extraction or column chromatography. This certainly warrants further investigation.

One argument favoring use of the digestion procedure is that it simultaneously extracts and saponifies the samples whereas an additional step is required for the Soxhlet extraction. It may be feasible to conduct the saponification in the Soxhlet pot thereby eliminating this disadvantage. However, if the analyst wishes to simultaneously analyze the sediment sample for chlorinated hydrocarbons such as DDT and for non-chlorinated hydrocarbons, then saponification must not occur before splitting the lipid extract in order to avoid chemical transformation of the DDT during saponification.

The results of Soxhlet extraction and subsequent hydrocarbon analyses for samples of surface sediments from three different depositional regimes are given in Table 11. The agreement of duplicate analysis for surface sediments of the continental shelf sediment station G187DG is well within the precision for the Narragansett Bay station C subsamples. However, agreement of the duplicate analyses of the deep sea abyssal plain surface sediment is not as good. Our investigation of the influence of different

Table 11. REPLICATE ANALYSES OF SEDIMENT SUBSAMPLES FROM THREE DEPOSITIONAL ENVIRONMENTS.

LOCATION	WATER DEPTH (METERS)	SEDIMENT TYPE	μg HYDROCARBON/g DRY WEIGHT f_1 (SATURATES)
Narragansett Bay (Station C) 41°33.4'N, 71°25.3'W	3.1	Sandy-Silt	147 ± 54 (5 analyses)
Hudson Trough (G187DG) 40°04'N, 73°28'W	78.0	Silt Clay	58.3 48.0
Abyssal Plain (K 19-4-9) 30°01'N,60°02'W	5252	Clay	1.2 2.9

sediment type and varying concentrations of hydrocarbons in the sediment on the precision of measurement is not yet complete.

The concentration of hydrocarbons in the surface sediments at station C in Narragansett Bay are close to that reported three years ago - 130-160 μg/g dry weight for fraction 1 and fraction 2 hydrocarbons (57) as compared to 202 and 265 μg/g dry weight for the latest analysis. We cannot be certain at this time whether this difference is a result of the slightly different methods of analysis or is a real increase in hydrocarbon concentration. This illustrates the need for the type of investigation of analytical methods we are pursuing.

Analyses of Sediment Samples Spiked with 10 ppm API No. 2 Fuel Oil - We have applied the Soxhlet extraction procedure to the analysis of station C surface sediments spiked with 10 μg API No. 2 fuel oil per gram dry weight of sediment. As expected and shown in Table 9, gravimetric quantification of the column chromatography fractions by weighing on the Cahn electrobalance did not detect the added No. 2 fuel oil in the presence of the base load of hydrocarbons.

Gas Chromatographic Analyses - The reproducibility of the gas chromatographic analyses of duplicates of fraction 1 hydrocarbons is demonstrated in Figures 27 and 28. The reproducibility of the gas chromatogram for the saturated fraction of the abyssal plain sediment hydrocarbons was similar. The main features of the gas chromatograms are:

i) The predominance of an unresolved complex mixture of alkanes and cycloalkanes with a wide molecular weight range. This is similar to that found in petroleum and is not predominant, if present at all, in recently biosynthesized hydrocarbons. The sources of these hydrocarbons are most

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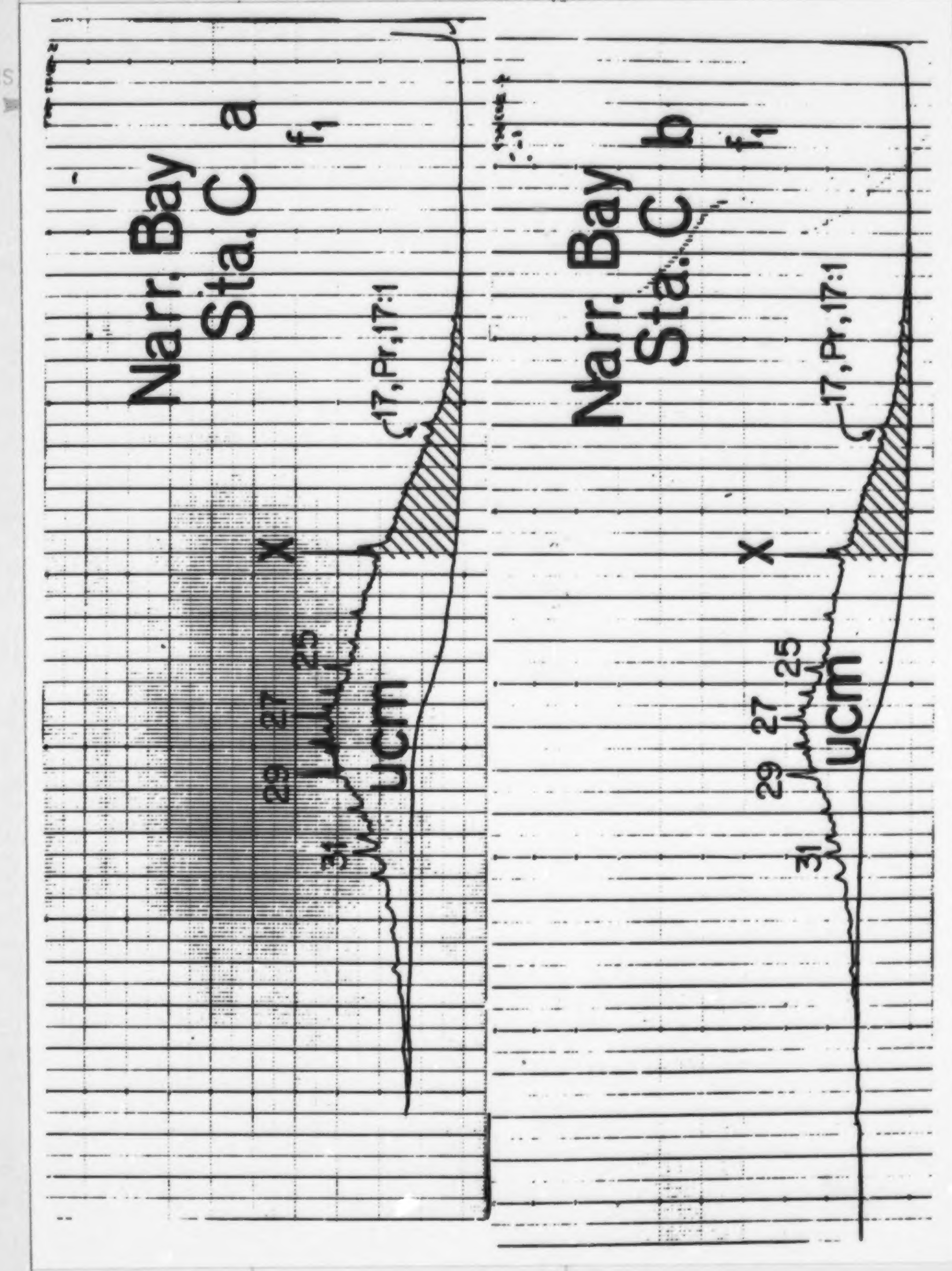

Figure 28. Gas chromatograms of alkanes, cycloalkanes, alkenes and cycloalkenes from duplicate extractions of surface sediments from Station C Narragansett Bay, Rhode Island.

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likely petroleum discharged to the marine environment via a combination of man's activities. The various factors bearing on this contention have been previously discussed. We think that for concentrations of hydrocarbons about an order of magnitude lower than that reported for station G187DG, the source of the unresolved complex mixture may be weathering of ancient sediments and transport and deposition of the sediments and their associated petoleum-like hydrocarbon assemblages to the surface sediments of the continental shelf and estuarine areas. In addition, at low concentration levels of hydrocarbons there may be some as yet undefined biosynthetic or biogeochemical diagenetic processes in surface sediments which produce such a hydrocarbon assemblage.

ii) The presence of the partially resolved n-C_{17}, pristane, C_{17} mono-olefin. These hydrocarbons are most likely of recent biosynthetic origin (see Introduction section).

iii) The presence of the heavier molecular weight n-C_{23} to n-C_{31} n-alkanes with an odd carbon predominance. Land plants and marsh grasses are probably sources for these n-alkanes.

iv) The presence of fully or partially resolved components in the n-C_{27} to n-C_{31} molecular weight range for station G187DG sediments which have been tentatively identified as triterpanes and steranes based on gas chromatographic retention data and gas chromatography-mass spectrometry. These compounds are probably present in station C sediment but are not well resolved in the presence of the higher concentration of unresolved complex mixture of cycloalkanes and alkanes. The origin of these compounds is probably a combination of biosynthesis and diagenesis.

v) The presence of partially resolved hydrocarbons indicated by the X in the gas chromatograms. The structures of these hydrocarbons are not known. Gas chromatograph-mass spectrometer analyses of the compounds after trapping from gas chromatography and before and after hydrogenation indicates that the major component of the mixture is a 25 carbon cycloalkene with an isoprenoid side chain. This will be discussed in section 9.

Regardless of the origin of the hydrocarbons in the surface sediments at station C and station G187DG, they represent present baseload concentrations and compositions. Station C is located in Narragansett Bay, an estuary which has substantial tanker traffic and effluent discharges (57). Station G187DG is on the continental shelf in the Hudson trough east-southeast of the New York Bight. It is adjacent to the proposed outer continental shelf drilling and production sites. It is also near sewage, sludge and dredge spoil dump sites, tanker routes, and could receive petroleum contaminated municipal and industrial effluent from the adjacent coastal area. Any determination of the concentrations and composition of hydrocarbons incorporated into these sediments as a result of an accidental spill or new nearby chronic releases of oil would have to take these present hydrocarbon concentrations and compositions into account.

A simple but necessary experiment was conducted to determine the feasibility of detection a low concentration of 10 ppm freshly spilled fuel oil incorporated into the station C sediments. The results of the gas chromatographic analyses are given in Figure 29 along with the gas chromatogram of the fraction 1 hydrocarbons of the API No. 2 fuel oil. A comparison of the gas chromatograms of Figures 28 and 29 shows that the presence of the 10 ppm spike of No. 2 fuel oil in the sediment sample is clearly detected. Obviously, higher concentrations of the fuel oil would be detected using similar procedures. However, the detection of lower concentrations of spiked fuel oil hydrocarbons, i.e. at the 1 ppm level will be more difficult. The detection of the presence of the No. 2 fuel oil in sediments by gas chromatography as was accomplished with the West Falmouth oil spill studies (65, 93, 94, 134) does not provide a quantitative measurement of low concentrations of the No. 2 fuel oil hydrocarbons. In the case of the West Falmouth oil spill it was evident for the sediments with the heaviest concentration of oil (1,000 μg/g dry weight sediment) that gravimetric determination of the hydrocarbons using a Cahn electrobalance or analytical balance provided a fair assessment of the No. 2 fuel oil concentration. However, the results previously reported in this paper clearly demonstrate that at concentrations of about 100 μg total hydrocarbons/gram dry weight of sediment the gravimetric determination cannot detect the presence of the No. 2 fuel oil at the 10 ppm concentration level. This applies to many of the sediment samples analyzed in the West Falmouth oil spill as well as at other oil spill sites (135). A good method of measurement is needed if correlations of acute and chronic biological effects at low concentration levels of No. 2 fuel oil are to be determined. Likewise, a method of measurement is necessary to provide a true assessment of the geographic extent of the spread of petroleum hydrocarbons from an oil spill site and the persistence of a given low concentration of oil in sediments over a period of time.

One method for measurement of petroleum hydrocarbons spiked to a marine lipid extract which has been used with some success is calibration of the flame ionization detector of the gas chromatograph with a standard mixture of n-alkanes followed by quantification of the gas chromatograph detector signal for the hydrocarbons from the sediments for the spiked and unspiked samples. We have conducted an initial test of this approach by quantifying the detector signal for the n-C_{12} to n-C_{20} molecular weight range of the fraction 1 hydrocarbons of the No. 2 fuel oil using planimetry. The gas chromatograms used were those shown in Figure 28 and the duplicate of the one at the top of Figure 29.

Agreement of the duplicate analyses is fair as is shown in Table 12. Recovery of the fraction 1 hydrocarbons from column chromatography of the API No. 2 fuel oil and evaporation to concentrate the sample are also given in Table 12. The lower boiling hydrocarbons in the fuel oil are lost during this procedure. The measurement of the No. 2 fuel oil hydrocarbons in the spiked sediment samples gives 2.9 μg hydrocarbons/g dry weight of sediment which is lower than the 4.9 μg/g dry weight predicted from the recovery experiments with the No. 2 fuel oil. This lower value is probably due to a more extensive loss of lower boiling hydrocarbons in the fuel oil spike due to the extensive handling during several concentration steps in the sediment analysis procedure.

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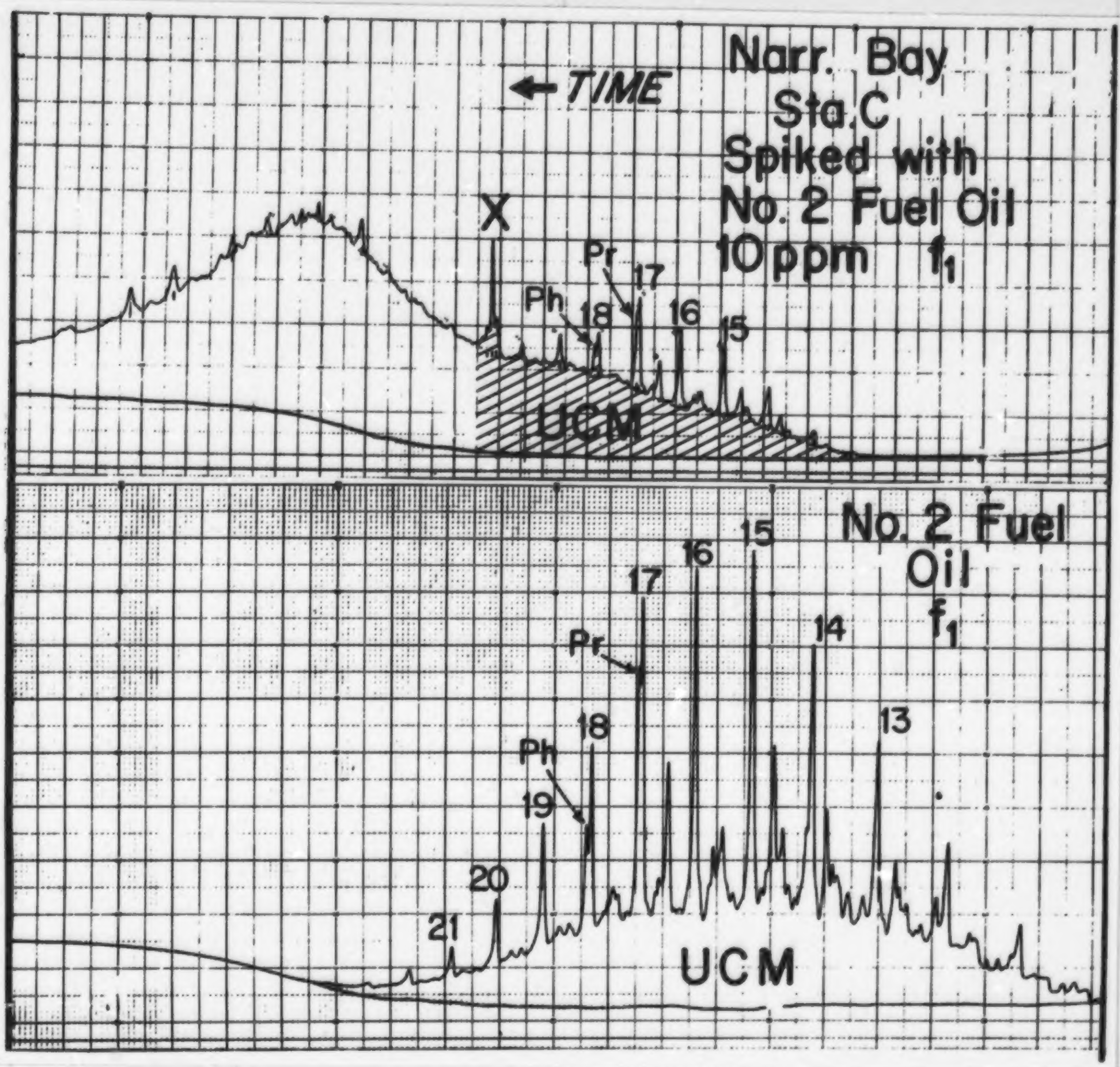

Figure 29. Gas chromatograms of alkanes, cycloalkanes, alkenes, and cycloalkenes of surface sediments Station C spiked w/API No. 2 fuel oil

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TABLE 12. GAS CHROMATOGRAPHIC QUANTIFICATION OF THE n-C_{12} TO n-C_{20} MOLECULAR WEIGHT RANGE OF HYDROCARBONS IN UNSPIKED AND FUEL OIL SPIKED SURFACE SEDIMENT.

		μg f_1 HYDROCARBON/g DRY WEIGHT SEDIMENT
Sediment, Not Spiked		1.6 1.7
	$\overline{\text{AV.}}$	1.6
Sediment, Spiked with 10 μg Fuel Oil/g Wet Weight Sample		5.4 3.4
	$\overline{\text{AV.}}$	4.4
Spiked-Unspiked		2.9
Recovery of API No. 2 Fuel Oil for Column Chromatography		4.3 (43%)

A comparison of the gas chromatograms in Figure 29 suggests that this is the case. Incorporation of an internal standard into the sediment sample prior to extraction would probably improve accuracy. This involves splitting the sample and analyzing subsamples with and without an internal standard in order to avoid masking information on the indigenous hydrocarbons and to correct for the interference of indigenous hydrocarbons with measurement of the internal standard.

We do not have gas chromatographic analysis of the fraction 2 and fraction 3 hydrocarbons to present at this time.

The limited data we have presented suggests that the methods as described above have a good potential for providing reliable estimates of fuel oil hydrocarbons in surface sediment samples. We realize that the experiments performed do not take into account alteration of the oil by weathering or degradation during and after incorporation into the surface sediments. However, if the methodology fails the simple tests we are applying then it has little possibility of working for the more difficult field test. Application of these methods to the field situation requires a surface sediment from the area prior to the oil pollution incident or a control station nearby which has a similar hydrocarbon composition and concentration as the oil spill area prior to the pollution incident.

We are certainly not yet at the point where we can formulate a standard method of analysis for petroleum hydrocarbons in sediments at the 10 ppm level. The analytical methodology to be tested is by no means limited to the methods we have discussed in this paper and we urge others in this field of research to pursue testing procedures such as these to investigate the efficiency, precision, applicability, and limitations of other methods of analysis. Without such testing, interpretation of the data collected during the studies of the input, fate, and effect of petroleum pollution in the marine environment will be unnecessarily restricted.

SECTION 7

INTERCALIBRATION OF ANALYSES OF HYDROCARBONS IN MARINE ORGANISMS

An important factor in field or laboratory studies is the precision, accuracy, and intercomparability of the data gathered using the same or different methods of analyses in different laboratories (1-3). We initiated a program of intercalibration of the analyses of hydrocarbons in cod liver oil spiked with petroleum compounds with funding from the Office for the International Decade of Ocean Exploration, National Science Foundation. We have continued this program with funding from this grant. We have reported on several phases of this intercalibration program (6, 39) and will not repeat those data and interpretations here.

As part of the program we distributed vials of cod liver oil spiked with a distillate cut of South Louisiana crude oil. We distributed the sample to 35 laboratories. Six laboratories reported data. All the others could not perform the analyses for a variety of reasons, including lack of time, funds, not possible to analyze sample, or did not know what to do.

The data from the six laboratories is given in Table 13. The agreement is remarkably good for the pristane. The agreement for the total of unresolved mixture hydrocarbons is also good. The estimate of petroleum compounds given by the UCM measure 363 μg/g cod liver lipid and the concentration of the South Louisiana crude oil spike of 371.8 μg/g cod liver lipid is very good.

Since we completed these intercalibrations several others under the auspices of the U.S. National Bureau of Standards and the Bureau of Land Management, Department of the Interior have been initiated or completed. Our program showed that it was possible and worthwhile to undertake these intercalibrations and that laboratories could attain reasonable agreement for certain types of hydrocarbon analyses.

TABLE 13. RESULTS OF IDOE-5 INTERCALIBRATION ANALYSES.

LABORATORY	PRISTANE CONCENTRATION μg/g COD LIVER LIPID	UNRESOLVED COMPLEX MIXTURE[e] μg/g COD LIVER LIPID
A	276[a]	349.9[a]
B	243.5[a]	396[c]
C	262[c]	385
	256	385
	272	613
	264	288
D	276[a]	350
E	272[d]	343[d]
	267	384
	270	367
F	238[a]	519.6[a]
Grand Ave. =	263 ± 13 s.d.	363 ± 102 s.d.
	4.9% r.s.d.	28% r.s.d.

Distillate cut of South Louisiana crude oil spiked at 371.8 μg/g cod liver lipid.

---

[a] Precision not reported.

[b] Not reported.

[c] Four analyses on four different dates.

[d] Three analyses on three different dates.

[e] Estimate of petroleum contamination.

SECTION 8

MASS FRAGMENTOGRAPHIC DETERMINATION OF AROMATIC HYDROCARBONS IN MARSH AND COASTAL SEDIMENTS

1. Introduction

Our knowledge of the composition and distribution of marine hydrocarbons has increased rapidly with the development of suitable extraction and isolation procedures (1-10), and application of instrumental techniques such as gas chromatography and mass spectrometry (130, 131, 139, 143). However, marine sediments and, by implication, other environmental samples, contain such an extremely complex assemblage of alkanes, alkenes, cycloalkanes, and aromatic hydrocarbons that quantitative determination of individual hydrocarbons by either gas chromatography or mass spectrometry is often difficult (2, 130, 131).

The use of combined gas chromatography-mass spectrometry in geochemical studies has generally been limited to structural identifications (9, 144-146). We present data here on the abundance of selected naphthalenes and phenanthrenes in coastal and marsh sediments, as determined by quantitative gas chromatography-mass fragmentography. These aromatic hydrocarbons were chosen for study because they are important potential indicators of petroleum contamination (1, 141-143), are toxic to some marine organisms (1, 147) and have been shown to persist in sediments up to one and one-half years after a No. 2 fuel oil spill (65, 94).

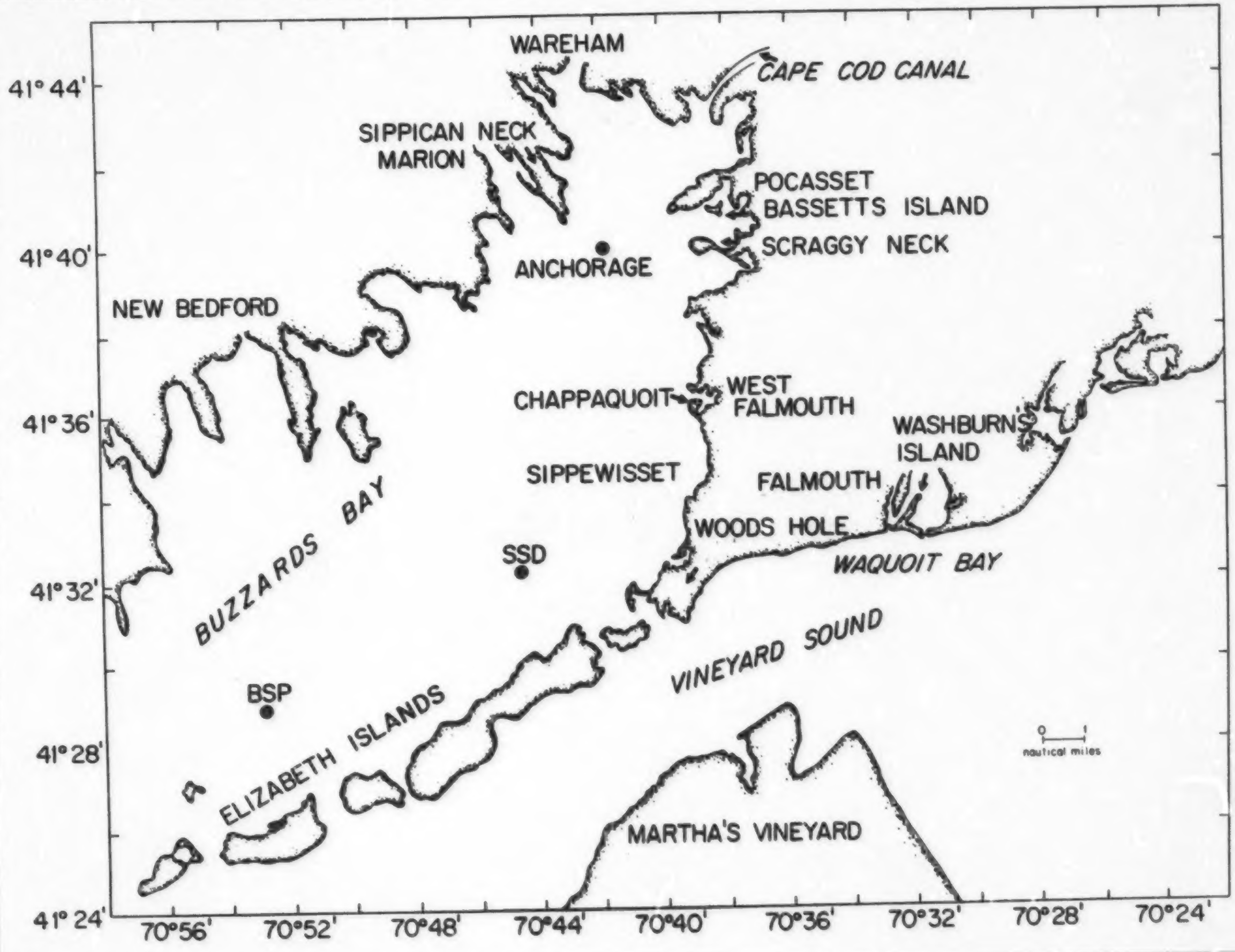
2. Experimental

Sampling

Sampling locations, as shown in Figure 30, included a station at the southern end of Buzzards Bay (BSP) and two stations within the vicinity of Winsor Cove where a small No. 2 fuel oil spillage occurred in October, 1974 from Bouchard Barge No. 65. Coastal sediments were collected with a 21 cm diameter sphincter corer. Marsh sediments were taken with a standard grab sampler or hand coring device.

Isolation Procedure

Aromatic hydrocarbons were isolated from the sediment samples using procedures previously described in section 6. Samples were Soxhlet extracted with 1:1 benzene-methanol and saponified in methanol-KOH-H_2O. The sulfur-free, non-saponifiable lipid fraction was then purified by column chromatography on 5% deactivated alumina-silica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CAPE COD CANAL
SIPPICAN NECK
MARION
POCASSET
BASSETTS ISLAND
SCRAGGY NECK
ANCHORAGE
NEW BEDFORD
CHAPPAQUOIT
WEST FALMOUTH
WASHBURNS ISLAND
SIPPEWISSET
FALMOUTH
WOODS HOLE
WAQUOIT BAY
BUZZARDS BAY
SSD
VINEYARD SOUND
BSP
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Figure 30. Sampling locations - Buzzards Bay, Massachusetts.

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gel. Two fractions were eluted from the column and collected. The column was first eluted with one column volume of pentane to give alkanes, cycloalkanes, alkenes, and cycloalkenes. A second fraction, containing primarily two and three ring aromatic hydrocarbons and the remainder of the alkenes and cycloalkenes was eluted with one column volume of 10 percent benzene in pentane, followed by one column volume of 20 percent benzene in pentane. Samples were then concentrated to a minimum volume on a rotary evaporator and taken up in 20-200 ul hexane for mass spectrometry analysis.

Instrumentation

The instrumentation consisted of a Finnigan 1015 quadrupole mass spectrometer equipped with an electron impact source. The spectrometer was interfaced to a Varian 1400 gas chromatograph via a single stage glass jet separator. Data acquisition and processing were controlled by a System Industries 150 data system.

Gas chromatography was performed using a 2 meter, 6 mm O.D., 1.8 mm I.D. glass column packed with 2 percent Apiezon L (pre-purified by column chromatography) on Chromosorb W (100/120 mesh). Injector and interface temperature were maintained at 250°C. The column oven was temperature programmed from 100°C to 270°C at 4°C/minute. Helium was used as the carrier gas at a flow rate of 25 ml/minute. Mass spectrometer conditions were as follows: Ion source, 500 μA ionization current at 70 electron volts; preamplifier gain, 10^{-7} amperes/volt; electron multiplier voltage, 2.2 kv; resolution, unit mass to 750 amu; scan conditions, 100-250 amu at 16 milliseconds/amu.

Data Collection and Treatment

The mass spectrometer was carefully calibrated using perfluoro-tri-n-butyl amine prior to each series of measurements in order to minimize errors due to instrument draft and mass defect. Since large variations in component concentrations were expected from sample to sample, the dynamic range of the detection process was maximized by operating the spectrometer in the IFSS mode (integration as function of signal strength), but in such a manner as to retain a nearly constant rate of scan. This was accomplished by using a base integration time of two milliseconds and requesting identical minimum and maximum allowable repeat counts before a threshold comparison was performed.

Volumes of column chromatographic isolates of sediment extract ranging from 0.5-1.5 ul were injected into the chromatograph with the spectrometer source filament and detector power supplies in standby mode. After elution of the solvent peak, the power supplies were reactivated and allowed to stabilize for 30 seconds before initiating the temperature program and data collection.

Upon termination of data collection, ion chromatograms for the molecular ions of specific aromatic hydrocarbons were constructed using the Tektronix 4010 display terminal, and the area under the chromatogram integrated. The limits of integration were determined from retention times for standards and through examination of the mass spectra to check for the correct fragmentation patterns and for possible interferences. Response curves for each aromatic component of interest were obtained by injecting aliquots of a standard aromatic hydrocarbon mixture of known composition and plotting integrated molecular ion current vs. amounts injected. The corresponding ion current integrals for the sample eluate were then compared with these to obtain the amount of each component in the sample. Instrument response was calibrated frequently due to unavoidable drift in the mass calibration and to the increasing tendency of the chromatographic column to irreversibly adsorb sample components, particularly the higher molecular weight components.

Calibration curves for the components tested (naphthalene, 1-methylnaphthalene, acenaphthene, 2,6-dimethylnaphthalene, 2,3,6-trimethylnaphthalene, 1-methylphenanthrene, and 3,6-dimethylphenanthrene) were linear over the concentration ranges shown in Figure 31. Sample eluates were appropriately diluted such that component concentrations fell within these ranges, although a linear response could probably be obtained over a much wider range.

Precision, as determined by multiple injections of naphthalene and 1-methylnaphthalene standards, was approximately 2-3 at the level of 50 ng material injected at 12% at the one nanogram level. This compares favorably to a quantitative gas chromatographic determination at these concentration levels. The detection limit for naphthalene (signal/noise ratio 2:1) is estimated to be 0.05 nanograms/gram dry weight sediment. Selected ion monitoring could be used to increase sensitivity. However, the number of components which can be determined simultaneously is reduced, and the opportunity to scan the data for interfering fragments is lost.

3. Results and Discussion

Analysis of Fuel Oils

Since a principal concern of this study was to determine the extent of petroleum contamination in local sediments, the applicability of the mass fragmentographic method was tested with two representative fuel oils. At present, the only suggested reference fuel oil for which compositional data is available is the American Petroleum Institute No. 2 fuel oil (148, 149). We have compared our determinations of combined C_1-substituted naphthalenes and C_1 and C_2-substituted phenanthrenes with the values previously reported for this oil (149). In addition, we have analyzed a sample of the Bouchard fuel oil involved in the Winsor Cove spillage of October, 1974. Compositional data are presented in Table 14. The data are very limited, but show acceptable agreement. The Bouchard No. 2 fuel oil is considerably less enriched in naphthalene content than the API reference fuel oil.

It is difficult to determine the accuracy of the overall method, partly because of large uncertainties in the extraction efficiencies (to be discussed below), and partly due to the complexity of the hydrocarbon assemblage encountered in the sediment samples. As expected, response

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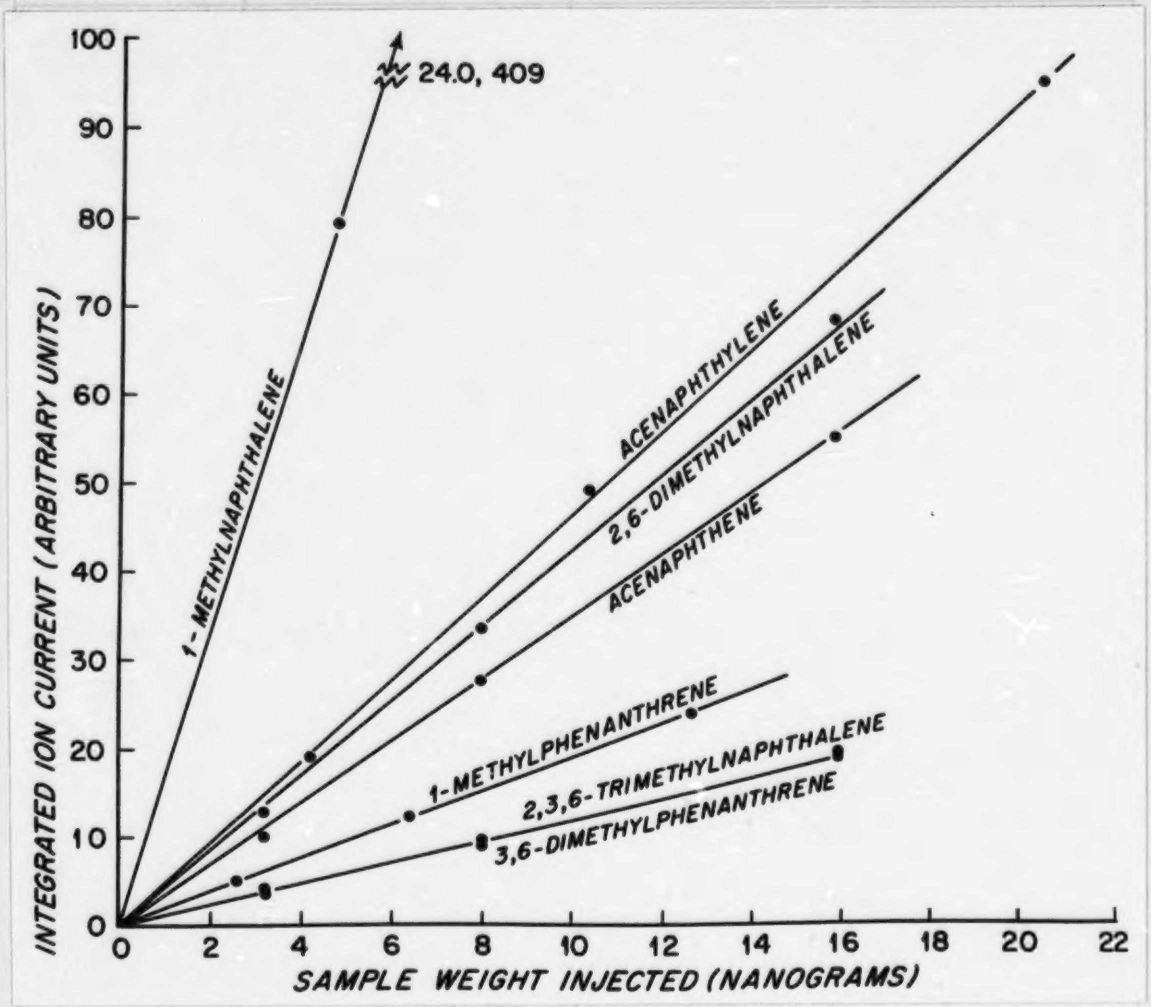

Figure 31. Calibration of aromatic hydrocarbon standards.

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TABLE 14. WEIGHT PERCENT OF SELECTED AROMATIC HYDROCARBONS IN FUEL OILS.

	NAPHTHALENE	C_1-NAPHTHALENES	C_1-PHENANTHRENES	C_2-PHENANTHRENES
API No. 2 fuel oil (present study)	—	2.0 ± 0.3	0.24 ± .02	0.23 ± .03
API No. 2 fuel oil (Warner, Ref. 23)	0.40	2.7	0.27	0.19
Bouchard No. 2 fuel oil	0.17 ± .01	0.95 ± .05	0.37 ± .02	0.33 ± .04

factors (Figure 31) vary significantly among classes of aromatic compounds and among individual isomers within a given compound class, and it becomes necessary to approximate the average instrument response using one or a few representative isomers as standards. We believe that for many applications, such as the present one, in which the relative isomer distributions reflected in the ion chromatograms from samples in a given core are remarkably similar, such an approximation is sufficient. A greater degree of accuracy could be attained by careful measurements on a large number of isomers using high resolution capillary columns.

Solvent Extraction/Column Chromatography Efficiencies

The efficiencies of the column chromatography and combined solvent extraction/column chromatography procedures have been measured to determine the extent to which the aromatic components of fuel oils are fractionated by the sample isolation procedures. Extraction efficiencies for recovery of total fuel oil by this method have been reported in section 6.

Column chromatography yields, as measured by mass fragmentographic analysis of both the Bouchard whole fuel oil and the equivalent aromatic fraction of the lipid extract, are presented in Table 15. Yields ranged from approximately 50 percent (naphthalene) to 95 percent (C_2-phenanthrenes).

Overall extraction efficiencies were determined by respiking three previously extracted sediments with aliquots of Bouchard fuel oil. These samples, two sandy sediments and one marsh sediment, were then taken through the entire analytical procedure, and the mass spectrometry determinations were then compared with those for the Bouchard whole fuel oil. Results are presented in Table 15. The low recoveries of the

TABLE 15. COLUMN CHROMATOGRAPHY YIELDS - BOUCHARD NO. 2 FUEL OIL.

COMPONENT	COLUMN CHROMATOGRAPHY	OVERALL EXTRACTION AND RECOVERY EFFICIENCY	
		SAND	MARSH
Naphthalene	56 ± 4%	6	4
C_1-Naphthalenes	76 ± 6%	30	12
C_1-Phenanthrenes	89 ± 5%	91	36
C_2-Phenanthrenes	96 ± 4%	94	37

naphthalenes in the sandy core are thought to arise from volatilization losses during rotary evaporation of the solvent, since recoveries for the less volatile phenanthrenes are similar to those measured for the column chromatography step alone. Recoveries for the spiked marsh sediment are considerably lower for both naphthalenes and phenanthrenes, suggesting that the nature and amounts of indigenous organic and inorganic material in sediments may be a strong factor influencing recoveries. Analyses of more sediment types are required to confirm this observation.

Aromatics in Marsh and Coastal Sediments

Results of analyses for naphthalenes and phenanthrenes in core sections from the station in Buzzards Bay (AST-4 BSP; see Figure 30) and from the Winsor Cove spill area are presented in Table 16 and 17, respectively. These data are uncorrected for the extraction and column chromatography yields. However, the uncorrected data are expected to be reasonably self-consistent, and it is possible to discuss certain important trends on this basis.

For the Buzzards Bay sediment core it is evident, for example, that the concentrations of phenanthrene and its C_1 and C_2-substituted homologs decrease with depth in the core by an order of magnitude. Pb-210 data obtained for this same core suggest an average sedimentation rate is 2.9 mm/year (150). This suggests that, in the absence of significant remobilization of biodegradation processes at depth, the input of these components has increased over the last two hundred years. Potential sources of these hydrocarbons have been discussed in detail elsewhere (see sections 4 and 9). However it is important to note that the concentration ratios of the C_1 and C_2 homologs relative to the unsubstituted phenanthrene (0.8:0.8:1.0, respectively) are considerably different from those expected from petroleum contaminants, in which the alkyl substituted aromatics are normally more abundant than the unsubstituted parent (141-143). The concentration ratios are relatively consistent from section to section within the core and appear to be more characteristic of pyrolytic sources (141-143).

TABLE 16. AROMATIC HYDROCARBONS IN BUZZARDS BAY CORE (AST-4 BSP) CONCENTRATIONS IN NANOGRAMS/GRAM DRY WEIGHT.

CORE SECTION	C_0-NAPHTH.	C_1-NAPHTH.	C_2-NAPHTH.	C_3-NAPHTH.	C_0-PHEN.	C_1-PHEN.	C_2-PHEN.
0-1 cm	0.2	1.4	5.7	14	NA	44	38
1-2 cm	0.1	0.3	2.4	10	34	27	28
8-12 cm	0.2	1.6	6.0	15	NA	31	29
20-24 cm	0.1	0.6	2.9	8.9	15	11	10
54-58 cm	ND	ND	ND	ND	3.7	2.9	2.8

NA - Not Analyzed.
ND - Not Detectable.

The distribution of naphthalenes in the core is more irregular, but also shows a decrease with depth. Comparison of the sections 0-1 cm and 20-24 cm suggest a more shallow gradient for the naphthalenes relative to the phenanthrenes. Although this could reflect water solubilization, it seems unlikely since the ratios of naphthalene homolog concentrations are roughly preserved in all core sections. Due to the skewed distribution of homolog concentrations arising from differing extraction efficiencies, the naphthalenes provide no additional information regarding potential sources. This could be overcome by employing an extract concentrating procedure which would recover the more volatile constituents.

The data from the Winsor Cove oil spill area illustrate some of the complexities of investigating the biogeochemical aspects of oil spills. The Winsor Cove sediments have been sampled a total of four times, beginning immediately after the spill and then at six month intervals. Only data for October, 1974 and May, 1975 are discussed here.

For the salt marsh location, the sediment burdens of the aromatic components measured (Table 17) decreased markedly in the initial six month period after the spill. Qualitative evidence for compositional changes of spilled petroleum during exposure and weathering, including depletion of the more soluble, low molecular weight components, has been cited previously in the West Falmouth oil spill incident (65, 94), which occurred in a nearby coastal area (Figure 30). In order to illustrate this on a quantitative basis, the data for the marsh sediments have been plotted in Figure 32 as concentration ratios (October, 1974/May, 1975) versus the specific aromatic hydrocarbon. A regular decrease in percentage reduction

TABLE 17. CONCENTRATIONS OF NAPHTHALENES/PHENANTHRENES IN WINSOR COVE SEDIMENTS (μg/GRAM DRY WEIGHT SEDIMENT).

	MARSH				SAND			
	OCT.		MAY		OCT.		MAY	
COMPONENT	DEPTH (cm)	CONC.	DEPTH (cm)	CONC.	DEPTH (cm)	CONC.	DEPTH (cm)	CONC.
Naphthalene	0-6	9.2	0-5	0.63	Surface Grab	ND	0-4	ND
							4-9	
	14-18	.024	15-20	.011			10-22	ND
					0-24	ND		
C_1-Naphthalenes	0-6	370	0-5	41	Surface Grab	.015	0-4	.050
							4-9	.002
	14-18	1.1	15-20	0.33			10-22	.0005
					20-24	ND		
C_2-Naphthalenes	0-6	1380	0-5	260	Surface Grab	0.23	0-4	
							4-9	
	14-18	5.1	15-20	1.5			10-22	
					20-24	ND		
C_3-Naphthalenes	0-6	3040	0-5	590	Surface Grab	0.84	0-4	
							4-8	
	14-18	12	15-20	3.4			10-22	
					20-24	.009		
C_1-Phenanthrenes	0-6	500	0-5	97	Surface Grab	0.25	0-4	0.79
							4-9	0.11
	14-18	2.2	15-20	0.94			10-22	.009
					20-24	.015		
C_3-Phenanthrenes	0-6	480	0-5	96	Surface Grab	0.25	0-4	0.70
	14-18	2.2	15-20	1.1			10-22	.006
					20-24	.012		

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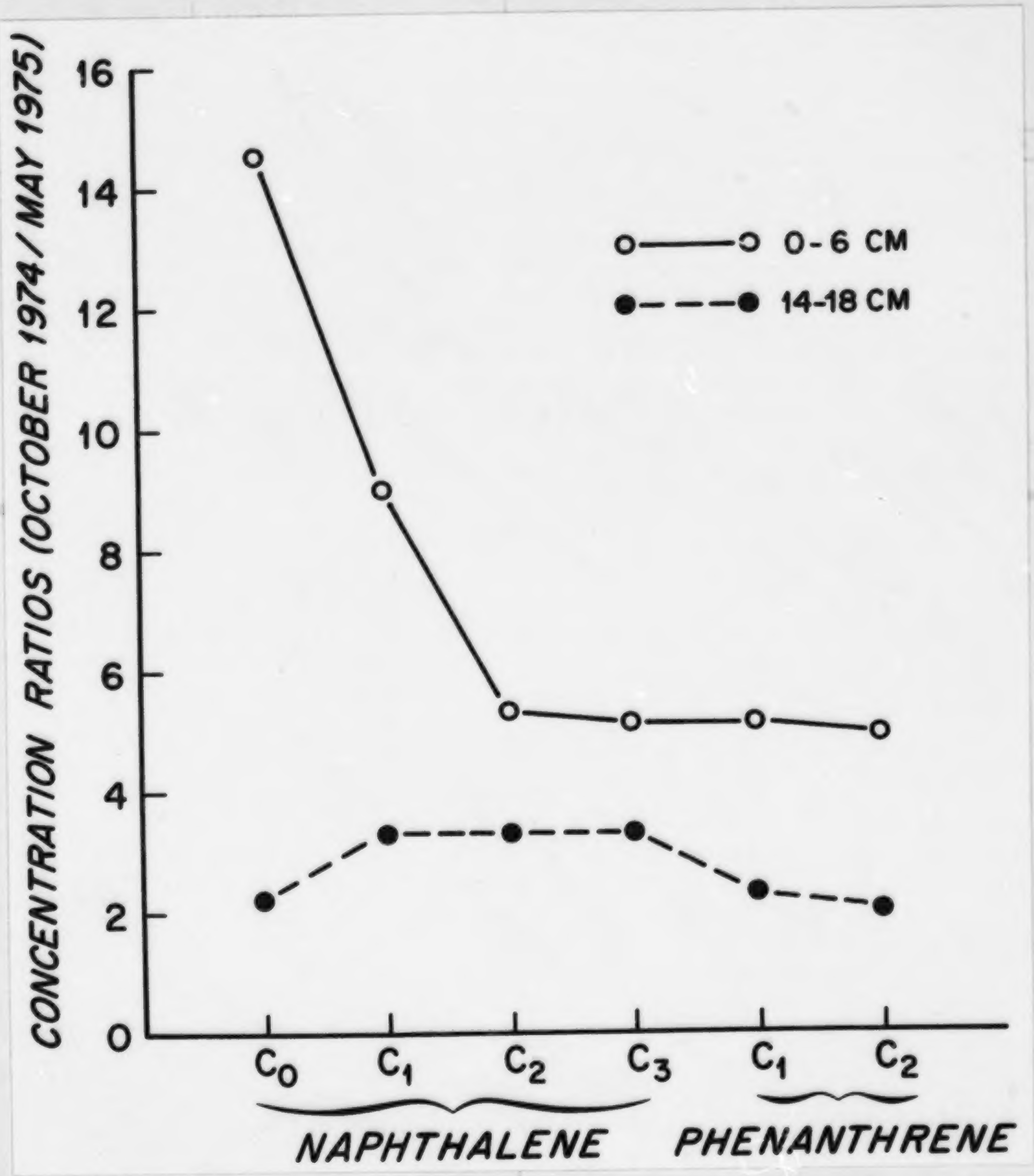

Figure 32. Reduction of aromatic hydrocarbon concentrations in marsh sediments, Windsor Cove, October 1974-May 1975.

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is evident for the series naphthalene to C_2-phenanthrenes in the 0-6 cm section, where water solubilization and dispersal are expected to be most intense. At the same time, the percentage reduction in the lower core section (14-18 cm) is roughly constant and is significantly lower.

In contrast to the marsh location, the aromatic hydrocarbons in the sandy core are much less concentrated and appear to have increased over the first six month sampling interval. Presumably, this is the result of a redistribution of oil from more heavily contaminated areas, as has been observed in the West Falmouth spill (65, 94). A more complete discussion of the biogeochemical studies of the spilled oil in the sediments is in preparation.

It is interesting to note that, for the sand cores, C_1 and C_2-phenanthrene concentrations in the 20-24 cm section (October) and the 10-22 cm section (May) are very similar to those found in the Buzzards Bay core at comparable depth and may derive from a source other than the fuel oil spillage. A strong possibility is urban air fallout as discussed in section 9.

4. Conclusion

The foregoing demonstrates the feasibility of using quantitative mass fragmentography for geochemical studies of the fate of selected aromatic hydrocarbons in marine sediments. While, in the present case, the composition of the organic matrix is extremely complex and it is probably feasible to quantitatively measure only the major components of the many overlapping homologous series present, the fundamental technique can and should be more widely applied to the study of geochemical processes. Combined gas chromatography-quantitative mass fragmentography offers a precision comparable to gas chromatography with the advantage of excellent selectivity. When coupled with glass capillary gas chromatography, quantitative mass fragmentography will be a powerful quantitative tool in geochemical and environmental investigations.

continental shelf. For one of these cores (AST-4 BSP), data on the Pb-210 geochronology exist (150) which can be correlated with the hydrocarbon data to reveal potential changes in hydrocarbon input over the time period represented in the core. To discriminate further among potential sources of the UCM, we have also analyzed concentrations of certain aromatic hydrocarbons as a function of depth in the AST-4 BSP core. We report here the results and interpretation of these and ancillary analyses.

2. Methods

The cores, obtained with a 21 cm diameter, 1 meter long sphincter corer (161) at the locations shown in Figures 30 and 33 were subsectioned and Soxhlet extracted as previously reported in section 6. The sulfur-free non-saponifiable lipid extracts were then separated into two main fractions using a column of alumina packed over silica gel (both 5% deactivated with water). The fraction 1 hydrocarbons which include alkanes, cycloalkanes, alkenes, and cycloalkenes were eluted with one column volume of pentane. Fraction 2 contained principally 2 and 3 ring aromatic hydrocarbons, alkenes, and cycloalkenes and is eluted with one column volume of 10% benzene in pentane followed by one column volume of 20% benzene in pentane. In this report we are primarily concerned with the fraction 1 hydrocarbons, and the phenanthrene in fraction 2.

Gas chromatographic analysis of the fraction 1 hydrocarbons was accomplished as previously described in section 6 on SCOT (Perkin Elmer Corporation) OV-101 or OV-1 columns of 25,000 plates, with the exception of the hydrocarbons from the Gulf of Maine core which were analyzed on an OV-17 column.

Concentrations of individual hydrocarbons in fraction 1 determined by comparing peak areas of the compounds to peak areas of internal standard n-alkanes (C_{14}, C_{18}, C_{22}, or C_{28}) were added to the sample at the time of extraction. Appropriate corrections for naturally occurring n-C_{14}, C_{18}, C_{22}, or C_{28} were applied where needed. The amount of hydrocarbons constituting the UCM was determined by integrating the total FID (flame ionization detector) signal minus the individual resolved peaks and converting this area to weight of hydrocarbon by comparison with the internal standard calibration factor. A standard mixture of the even carbon n-alkanes (C_{12} to C_{30}) was analyzed during the same day to obtain correction factors for possible losses of higher molecular weight hydrocarbons up to C_{30} due to non-ideal separation behavior of the GC column. The reproducibility of the analysis is estimated to be ± 37% relative standard error from the duplicates in Table 1 and by the analyses of other silt-clay and sandy-silt sediments (see section 6).

The fraction 1 hydrocarbons from several core sections were further separated by thin-layer chromatography on 5% $AgNO_3$ impregnated silica gel TLC plates (Supelco, Inc.) to isolate the alkenes and cycloalkenes from the saturated hydrocarbons. After development in pentane, the alkane + cycloalkane bands were visualized by spraying co-chromatographed standards with bromophenol blue and exposing to NH_3 vapors. The alkane and cyclo-

SECTION 9

HYDROCARBONS IN CORES OF NORTHWESTERN ATLANTIC COASTAL AND CONTINENTAL MARGIN SEDIMENTS*

1. Introduction

Surface sediments collected from a large number of sites on the continental margins, in coastal and estuarine areas, and from rivers and lakes, have been analyzed for hydrocarbon distributions during the past several years (30, 57, 72, 90, 93, 94, 144, 152-158). Some of these recent investigations have noted the complexity and wide molecular weight range of the hydrocarbons found in many of these sediment samples. In particular, gas chromatographic analyses on either medium resolution packed columns or high resolution capillary columns have frequently revealed the presence of a broad unresolved signal in the chromatograms. This signal, which has been called the "hump" (144), results from the co-elution or overlapping elution of a complex mixture of hydrocarbons (2-5). This signal is referred to as the unresolved complex mixture signal (UCM) here.

There are several possible sources for such a complex mixture of hydrocarbons (2, 3, 5, 144). In particular, spilled oil has been advocated as the likely source of this mixture, since it is known that a complex assemblage of cycloalkanes and branched cycloalkanes remains after substantial weathering and bacterial attack of oil (83, 97, 157, 159). Thus, the presence or absence of the UCM signal in a gas chromatogram may be a clue to the presence or absence of petroleum pollution and a means of estimating the quantity present. Additional impetus for investigation of the origin and distribution of this complex mixture is that it may provide insight into the geochemistry of the alkanes and cycloalkanes in Recent sediments.

A preliminary investigation of three sections from a core in Buzzards Bay, Massachusetts, revealed some interesting changes in the unresolved complex mixture (UCM) hydrocarbon concentration between surface (0-2 cm) and a deeper (70-72 cm) section. The concentration decreased by a factor of six (159). We therefore postulated that Buzzards Bay sediments contain a historical record of chronic oil release in this region. In order to investigate this possibility, we have measured hydrocarbon concentrations in core sections from samples collected at two stations in Buzzards Bay, a site in the Gulf of Maine, and a station in the Hudson Canyon on the continental shelf. For one of these cores (AST-4 BSP), data on the Pb-210 geochronology exist (150) which can be correlated with the hydrocarbon data to reveal potential changes in hydrocarbon input over the time period represented in the core. To discriminate further among potential sources of the UCM, we have also analyzed concentrations of certain aromatic hydrocarbons as a function of depth in the AST-4 BSP core. We report here the results and interpretation of these and ancillary analyses.

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2. Methods

The cores, obtained with a 21 cm diameter, 1 meter long sphincter corer (161) at the locations shown in Figures 30 and 33 were subsectioned and Soxhlet extracted as previously reported in section 6. The sulfur-free non-saponifiable lipid extracts were then separated into two main fractions using a column of alumina packed over silica gel (both 5% deactivated with water). The fraction 1 hydrocarbons which include alkanes, cycloalkanes, alkenes, and cycloalkenes were eluted with one column volume of pentane. Fraction 2 contained principally 2 and 3 ring aromatic hydrocarbons, alkenes, and cycloalkenes and is eluted with one column volume of 10% benzene in pentane followed by one column volume of 20% benzene in pentane. In this report we are primarily concerned with the fraction 1 hydrocarbons, and the phenanthrene in fraction 2.

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Concentrations of individual hydrocarbons in fraction 1 determined by comparing peak areas of the compounds to peak areas of internal standard n-alkanes (C_{14}, C_{18}, C_{22}, or C_{28}) were added to the sample at the time of extraction. Appropriate corrections for naturally occurring n-C_{14}, C_{18}, C_{22}, or C_{28} were applied where needed. The amount of hydrocarbons constituting the UCM was determined by integrating the total FID (flame ionization detector) signal minus the individual resolved peaks and converting this area to weight of hydrocarbon by comparison with the internal standard calibration factor. A standard mixture of the even carbon n-alkanes (C_{12} to C_{30}) was analyzed during the same day to obtain correction factors for possible losses of higher molecular weight hydrocarbons up to C_{30} due to non-ideal separation behavior of the GC column. The reproducibility of the analysis is estimated to be ± 37% relative standard error from the duplicates in Table 1 and by the analyses of other silt-clay and sandy-silt sediments (see section 6).

The fraction 1 hydrocarbons from several core sections were further separated by thin-layer chromatography on 5% $AgNO_3$ impregnated silica gel TLC plates (Supelco, Inc.) to isolate the alkenes and cycloalkenes from the saturated hydrocarbons. After development in pentane, the alkane + cycloalkane bands were visualized by spraying co-chromatographed standards with bromophenol blue and exposing to NH_3 vapors. The alkane and cyclo-

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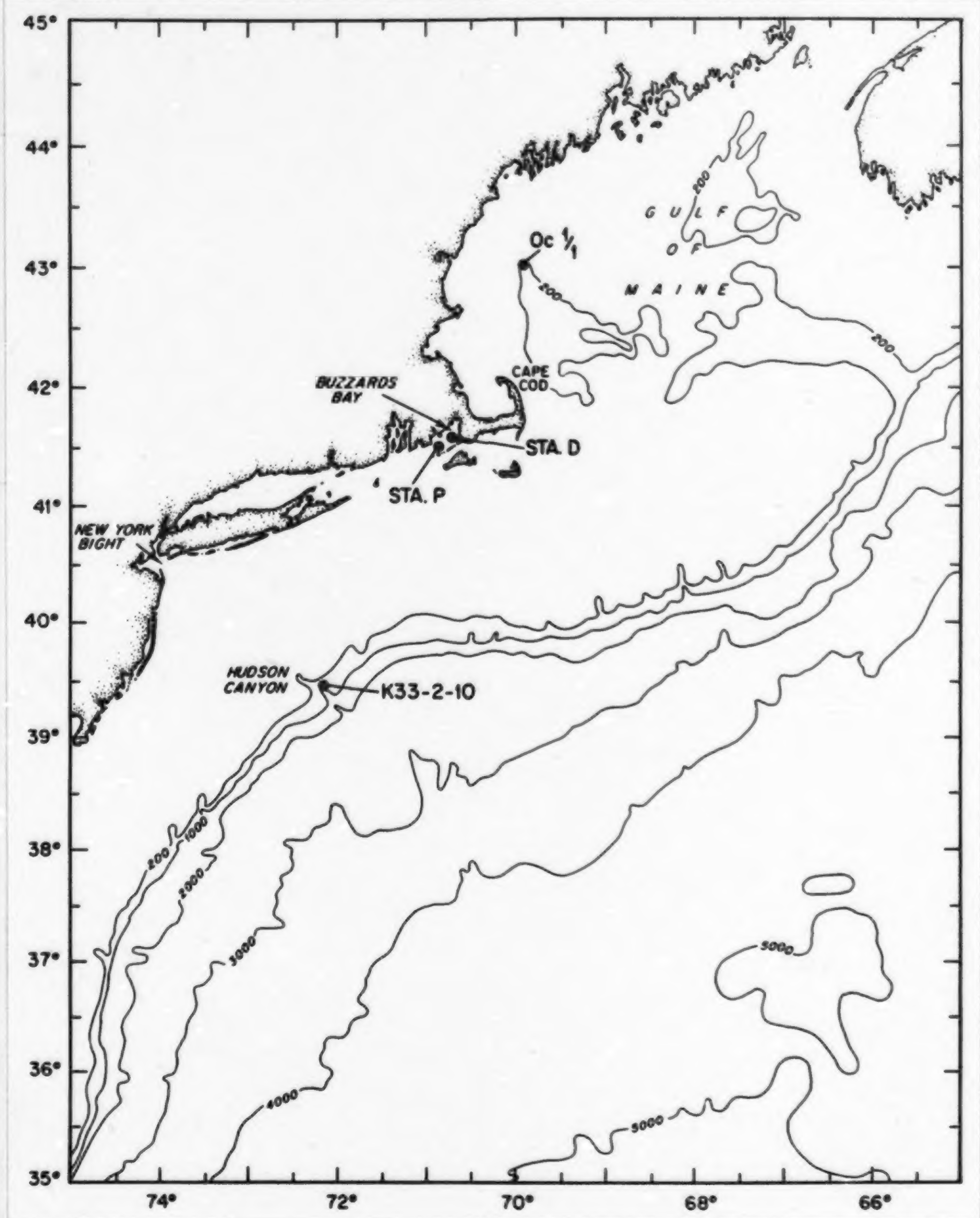
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STA. P
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HUDSON CANYON
K33-2-10
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Figure 33. Core sampling locations in the western North Atlantic.

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alkane bands were scraped from the plate and extracted with three washes of 2 to 3 ml of pentane:benzene (1:1) in a centrifuge tube capped with a Teflon lined screw cap. The hydrocarbons were then reanalyzed by gas chromatography to discern if there was any change in the mixture of hydrocarbons.

Concentrations of phenanthrene and its C_1 and C_2 alkyl-substituted homologs were determined by a quantitative mass fragmentographic method described in section 8. Known aliquots of the fraction 2 column chromatography eluates were subjected to combined gas chromatography-mass spectrometry using a Finnigan 1015 quadrupole mass spectrometer coupled to a Varian 1400 gas chromatograph. Response curves for the C_0, C_1, and C_2 substituted phenanthrenes were constructed using the integrated molecular ion currents obtained by injection of a standard mixture of aromatic hydrocarbons. The corresponding ion currents for the phenanthrenes in the sample eluates were then integrated, and the amount of each component calculated using the calibration curves. Determination of selected naphthalenes and phenanthrenes in the standard API No. 2 fuel oil by this technique has shown agreement within experimental error with values reported for this oil.

Two predominant compounds of unknown structure were isolated by preparative gas chromatography from 2 3.6 meter, 3 mm o.d. stainless steel packed column of 12.5 FFAP on Chromosorb W, AWS, using procedures described by Blumer et al. (32). Portions of the trapped compounds were hydrogenated over pre-reduced platinum (32). Retention indices of the compounds and their hydrogenated derivatives were obtained by analysis on SCOT OV-101 columns (Perkin Elmer Corp.) as previously described and on a packed Apiezon L column (32). A Varian Model 1440 GC equipped with a flame ionization detector and a Varian Model 1740 GC equipped with both a flame ionization and a flame photometric sulfur detector were used for the gas chromatographic analyses. Mass spectra of the isolated compounds and the hydrogenated derivatives were obtained on the GC-MS system. The compounds were introduced via the probe inlet using temperature programming from ambient to 150°C and the application of two different ionization potentials, 20 and 70 eV.

Organic carbon was determined as described in (150).

3. Results

The gas chromatograms for the core sections from Station AST-4, BSP are given in Figure 34. These chromatograms, along with the data presented in Table 18 show clearly that the concentrations of total hydrocarbons and UCM hydrocarbons decrease from surface sections to bottom sections of the core. In contrast, C_{23} to C_{31} n-alkanes do not decrease in concentration with increasing depth (Table 18 and Figure 34). The n-C_{29} concentration is given in Table 1 as a representative of this group of n-alkanes. Many of the chromatograms also indicate the presence of a group of related olefinic hydrocarbons of unknown structure with retention indices near 2000-2100, which decrease in concentration with increasing depth (Figure 34). This decrease is paralleled by a decrease in

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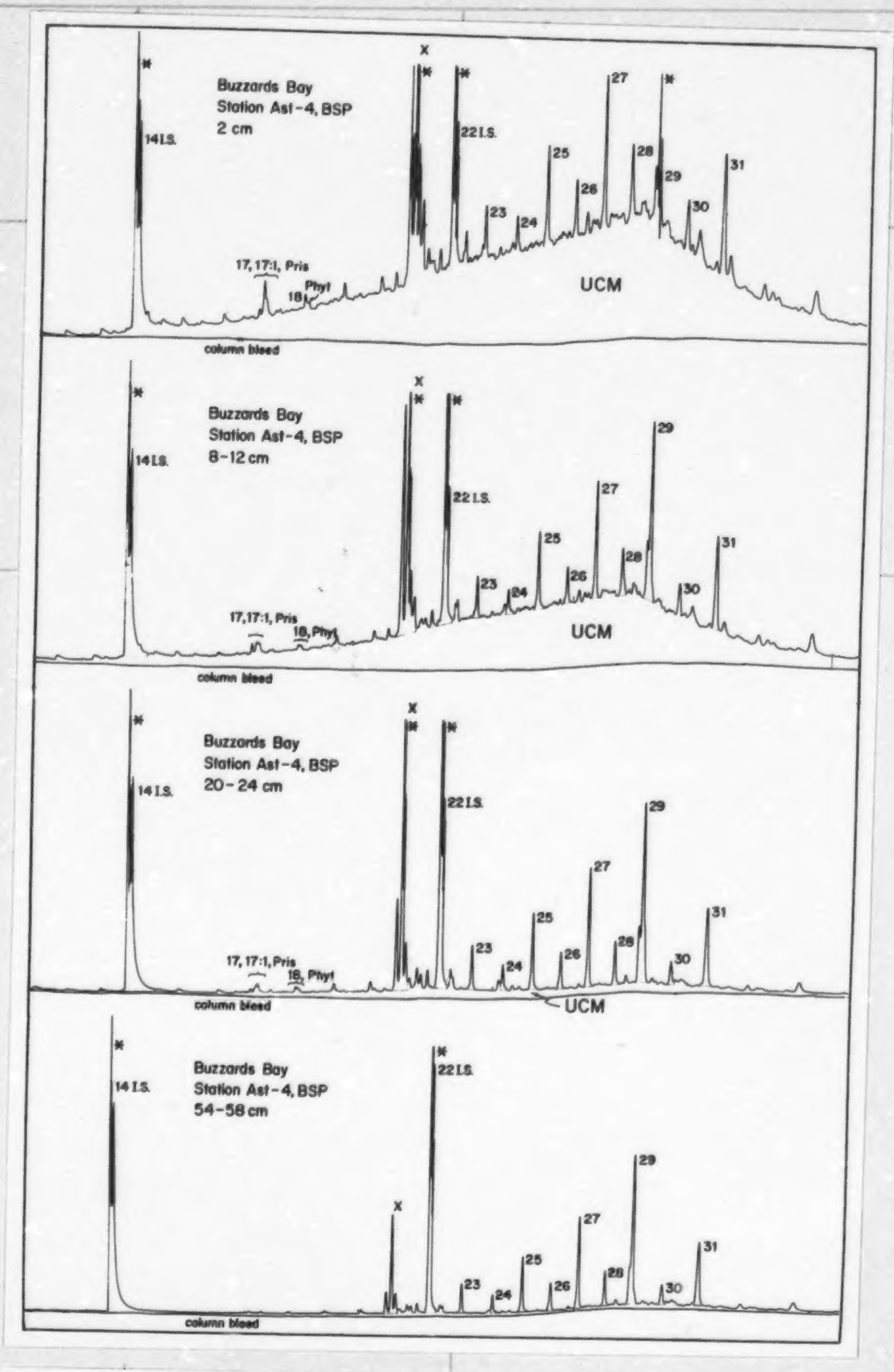

Figure 34. Gas chromatograms of alkanes, cycloalkanes, alkenes and cycloalkenes Station P Buzzards Bay core sections.

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TABLE 18. HYDROCARBONS IN BUZZARDS BAY, MASSACHUSETTS SEDIMENTS.

SAMPLE	ORGANIC CARBON ng/g DRY WT.	GRAVIMETRIC (µg/g DRY WT.)		GC FID RESPONSE (µg/g DRY WT.)		
		f_1	f_2	UCM	UNKNOWNS[a]	n-C_{29}
STATION P						
AST-4		November, 1974				
0-1 cmA	NA[b]	71	18	64	7.3	0.9
0-1 cm	NA	75	13	84	16.3	1.0
1-2 cm	23.8	110	39	105	5.0	0.9
8-12 cm	15.5	63	50	44	2.5	0.8
20-24 cm	16.3	21	17	12	2.4	1.1
54-58 cm	11.7	8	11	5.2	0.5	1.0
58-62 cmA	15.3	10	5	6.2	0.3	0.9
58-62 cmB	NA	8	2	--------Not Analyzed------		
AST-5		August, 1975				
0-4 cm	NA	79	46	63	4.2	0.8
20-24 cm	NA	17	15	15	1.2	0.8
38-42 cm	NA	17	20	15	1.3	0.6
STATION D						
AST-1		August, 1973				
0-2 cm	NA	NA	NA	60		0.7
2-4 cm	NA	NA	NA	55		1.0
70-72	NA	NA	NA	9.5		0.7

---

[a] Some of unknown olefins - compounds 1 and 2.

[b] Not analyzed.

the concentration of the C_{17} mono-olefin (Figure 34). The concentrations of phenanthrene and its C_1 and C_2 homologs are given in Table 19. There are lower concentrations in the AST-4 Station P core with increasing depth. The average ratios of the C_1 and C_2 homolog concentrations relative to the unsubstituted phenanthrene are 0.8:0.8:1.00, respectively, and are consistent throughout the core.

As a point of interest and comparison, the PCB and DDE concentrations in fraction 1 and fraction 2 were determined according to the procedures of Harvey and Steinhauer (162) for three sections of the AST-4 Station P core. Concentrations were below the levels of our blanks in the lower sections (Table 18).

There are five or six olefins of unknown structure present in the sediments. They elute from OV-101 columns with retention indices between 2000 to 2200 (Figure 34). Two of the principal components, compound 1 and compound 3 in order of elution from FFAP columns, have been isolated and investigated in some detail. Mass spectra and retention indices of these compounds and their hydrogenation products are given in Figures 35 and 36. The response of the flame photometric detector compared to that of the flame ionization detector response clearly ruled out the presence of sulfur in these two components. In addition, IR spectra suggested the absence of carbonyl functionality, as expected from consideration of our isolation procedure. The mass spectra of compound 1 suggest a $C_{25}H_{48}$ compound with at least one double bond, as indicated by the parent ion shift for the hydrogenated molecule. Several predominant even m/e fragments are present in the spectrum of the hydrogenated compound,

TABLE 19. HYDROCARBONS AND CHLORINATED HYDROCARBONS IN STATION P CORE SECTIONS.

SECTION	AVERAGE TIME OF DEPOSITION	UCM (µg/g)	PHENANTHRENES (ng/g) C_0	C_1	C_2	PCB (ng/g)	DDE (ng/g)
0-1 cm		74	NA	44	38	123	2.1
1-2 cm		105	34	27	28	NA	NA
8-12 cm	1940	44	NA	31	29	NA	NA
20-24 cm	1900	12	15	11	10	None	None
54-58 cm	1790	5.2	3.7	2.9	2.8	NA	NA
58-62	1780	6.2	NA	NA	NA	None	None

NA - Not Analyzed.

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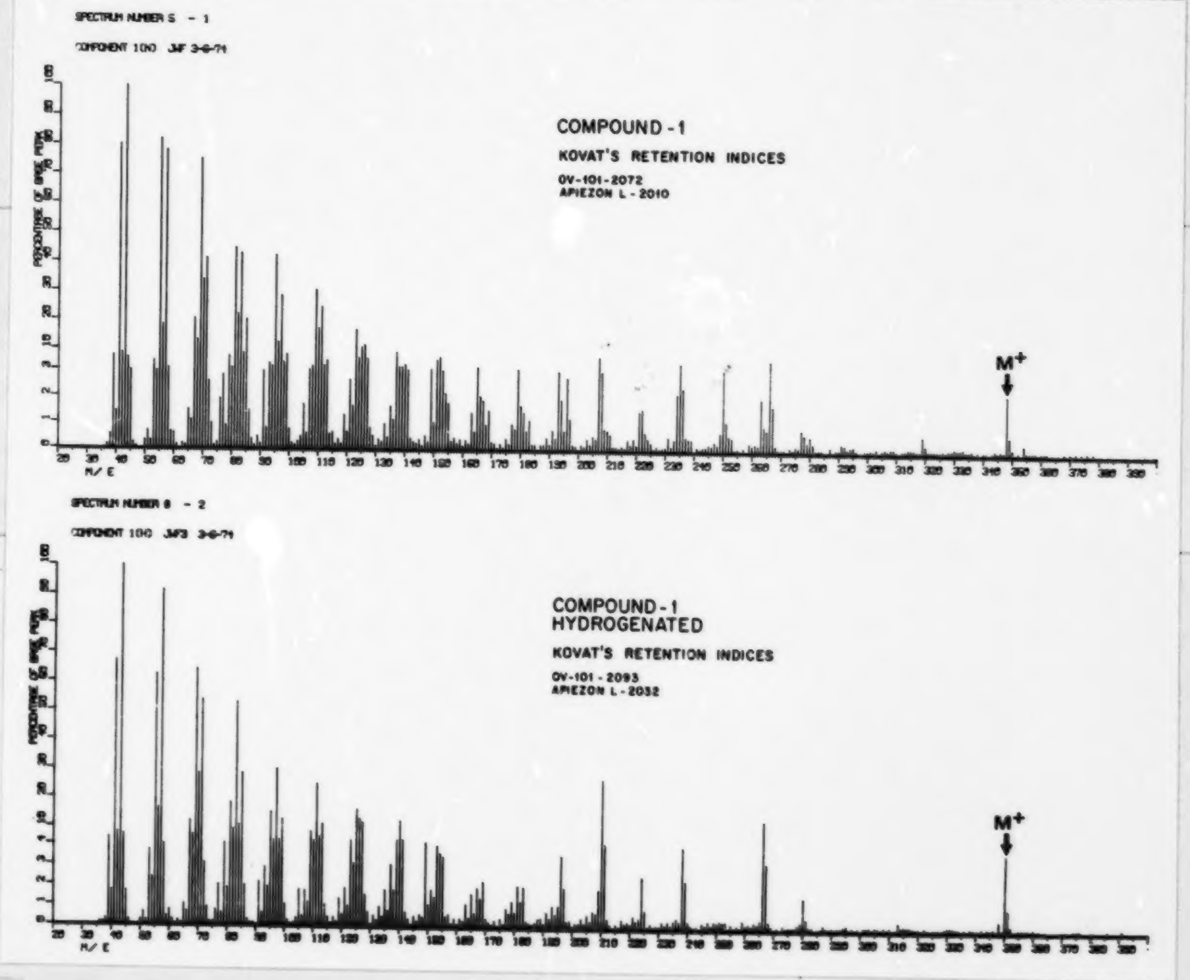

Figure 35. Mass spectra of unknown compound No. 1 and hydrogenation product.

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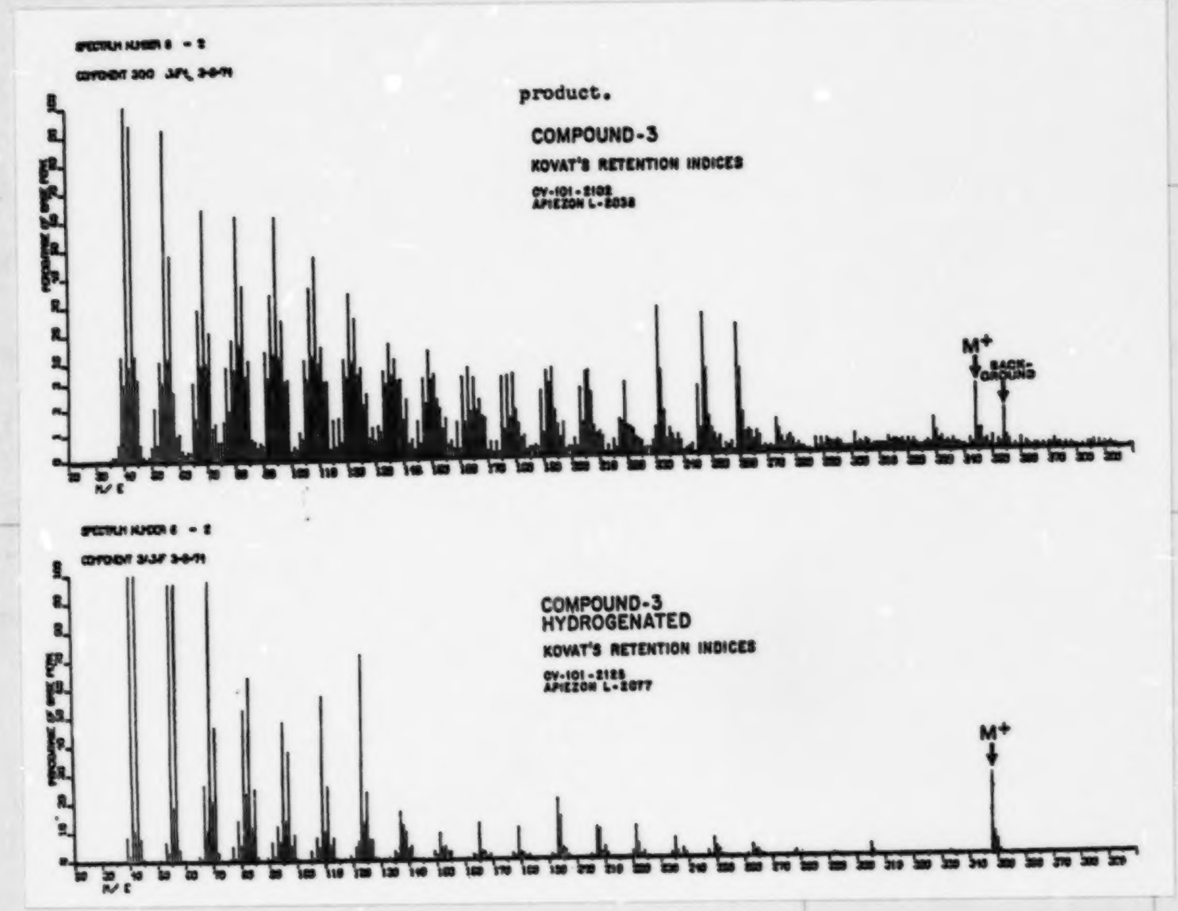

Figure 36. Mass spectra of unknown compound No. 3 and hydrogenation product.

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suggesting rearrangements of a cycloalkane leading to the observed spectra. The hydrogenated compound must contain one ring. The mass spectra of compound 3 before and after hydrogenation indicate that this compound is also a 25 carbon cycloalkene. The mass spectrum of the hydrogenated compound 3 requires two rings or a ring with an olefinic bond. The olefinic bond, if present, would have to be tetrasubstituted within the ring to have survived hydrogenation by our procedure. The mass spectra of compound 3 and its hydrogenation product are consistent with the presence of a branched side chain. Further structural elucidation by NMR, wet chemistry, and derivatization to locate the olefinic bond positions is needed to provide the structural proof of these compounds. Similar olefinic hydrocarbons of unknown structure, most likely the same ones as we have found here, have been reported for other surface sediments in coastal areas (5, 57, 91).

The decreasing concentration of the olefinic compounds with increasing depth led us to suspect that the major components of the UCM signal were olefinic in character, and therefore would decrease in concentration with depth due to the labile and reactive nature of the olefinic bond. Accordingly, we analyzed hydrocarbons from two representative sections of the Buzzards Bay core Station P by $AgNO_3$-TLC as described in the methods section. Reanalysis of the cycloalkane-alkane fraction isolated from TLC showed no significant difference in the concentration of the UCM hydrocarbons, but did show the absence of the unknown compounds and the C_{17} monoene as would be expected.

The hydrocarbon concentrations for sections of a core from Station D in Buzzards Bay are given in Table 18 and similar data for cores from the Gulf of Maine and Hudson Canyon are given in Table 20.

4. Discussion

The depth distribution of hydrocarbons in Station P sediments is not unique to that area. A few measurements at Station D in Buzzards Bay show the same general trends. The more detailed and comprehensive studies of Wakeham (163) and Wakeham and Carpenter (164) in Lake Washington document the widespread nature of this type of depth distribution in sediments within a given geographical area. Walker et al. (157) reported a similar depth distribution for Baltimore Harbor sediments. However, the depth distribution of hydrocarbons in the core from the Gulf of Maine (Table 20) shows no pronounced difference between the surface and deep section within analytical error, although the trend is the same as for Buzzards Bay cores. The lower concentration of the UCM in the surface sediment may be due to increased distance from the source(s). Another possibility is deeper bioturbation relative to the Buzzards Bay stations resulting in a larger amount of older material mixing with recently deposited material and diluting the recent input. We hope to investigate these possibilities in more detail.

The top sections of the Hudson Canyon core (Table 20) probably represent continental shelf or Hudson Channel sediment transported to the region by turbidity flows or bottom currents. These sediments with some

TABLE 20. HYDROCARBONS IN CORES OF SEDIMENTS FROM THE GULF OF MAINE AND HUDSON CANYON.

SAMPLE	ORGANIC CARBON mg/g DRY WT.	GRAVIMETRIC (μg/g DRY WT.)		GC FID RESPONSE (μg/g DRY WT.)		
		f_1	f_2	UCM	UNKNOWN OLEFINS	n-C_{28}
Oceanus 1/1	GULF OF MAINE	November 20, 1975		214 Meters Water		
0-1 cm	19.4	40	17	12.7	0.6	0.4
37-41 cm	NA	19	13	6.4	0.2	0.5
85-88 cm	15.6	9	15	5.1	0.1	0.5
K33-2-10	HUDSON CANYON	October 6, 1973		986 Meters Water		
0-8 cm	31.9	53	6.4	73	NA	1.5
8-20 cm	30.3	47	5.4	52	NA	1.3
20-40 cm	32.1	33	NA	19	NA	1.1
90-94 cm	26.3	5.6	2.0	0.5	NA	0.0

NA - Not Analyzed.

associated pollutant hydrocarbons overlie those deposited much earlier which do not contain the higher concentrations of the complex mixture of hydrocarbons.

We will focus most of our discussion on the data from core AST-4 Station P. The average sedimentation rate of this core has been established from the Pb-210 geochronology record to be 2.9 mm/year (150). Aside from the bioturbated upper 3 to 4 cm, the Pb-210 activity vs. depth record shows only minor disturbances which seem to occur at times in the record when severe storms passed through the area.

We do not believe that it is possible at present to make a quantitative assessment of the contribution of all the potential sources of the complex mixture of alkanes-cycloalkanes and aromatic hydrocarbons for the concentation levels present in these sediments. However, we will discuss the principal processes which might act on hydrocarbons deposited in modern sediments and the various sources of hydrocarbons with the intent of establishing the likely causes of the observed depth distribution. If the input, whatever the source, was constant over the time interval sampled by the core, then a mechanism(s) is required to provide the observed depth distribution of the UCM hydrocarbons. There are three possible processes which could be involved: (i) biodegradation at depth after initial burial, (ii) chemical reactions after burial, (iii) and/or remobilization of hydrocarbons at depth and movement to the surface sediments.

Biodegradation seems unlikely given the vast literature (1, 27, 66) which shows that n-alkanes are the biochemically more labile of the alkane-cycloalkane hydrocarbons. The depth distribution of the 23-31 n-alkanes remains essentially constant. Thus, unusual microbial processes are required for the UCM to be degraded and the n-alkanes to remain essentially untouched. One possibility that cannot yet be ruled out is that the complex mixture of alkanes and cycloalkanes is present in more easily attacked sediment particles than are the n-alkanes (150). Chemical reactions of organic matter at depth in the sediment with the resultant entrapment of hydrocarbons in humic or fulvic acids has been considered as a possibility for the decreasing concentrations of hydrocarbons with increasing depth. Such entrapment might prevent extraction by the Soxhlet procedure. We have treated the sediment remaining after Soxhlet extraction with alkaline digestion according to the procedure described in section 6. This treatment extracts some fulvic and humic material and should release some entrapped hydrocarbons. No more than 10 additional hydrocarbons were extracted and this amount cannot account for the lower concentrations of Soxhlet extracted hydrocarbons in the deeper core sections. We think that purely chemical transformation of alkanes to other compounds at depth in the cores is unlikely based on the extensive investigations of the long term stability of alkanes in deeply buried sediments (167).

One might question the plausibility of mobilization and redistribution of hydrophobic molecules such as hydrocarbons once they are absorbed onto sediment particles. However, Meyers and Quinn (101) have shown that the solubilization and accommodation of hydrocarbons in seawater are also dependent on the type of hydrocarbon and the organic matter present. When both of these investigations are considered, we have to allow that a complicated series of interactions between pore waters and sediment may preferentially mobilize certain hydrocarbons to the surface sediments. Their accumulation in surface sediments could be the result of mobilization at depth due to geochemical reactions or properties of anoxic deeper sediments which are not operative or present in oxic surface sediments. Our knowledge of the extent of remobilization of hydrocarbons at depth in Recent sediments is scanty and requires investigation. This knowledge is important for geochemical and environmental considerations. For example, the distribution of geochemical "marker" compounds (9, 144, 168) in sediments may be influenced by such remobilization processes. The fate of pollutant hydrocarbons incorporated into sediments and their availability to the benthos would be markedly influenced by remobilization phenomena. Thus, measurements of hydrocarbon concentrations and distributions in interstitial water are needed to determine if there is a flux from deeper sediments to the oxic surface sediments.

Next we will consider the various sources of hydrocarbons in the sediments and the possibility of each source being the predominant contributor of the UCM hydrocarbons.

(i) Biosynthesis: The available analyses in the literature rule out phytoplankton, land plants, benthic algae, and marine animals as predominant sources of the unresolved complex mixture of hydrocarbons (2). Bacteria and yeasts cannot be ruled out, based on analyses of cultures, since most were grown and analyzed in order to invesigate hydrocarbons other than the UCM (2).

Other evidence suggests that biosynthesis is not the predominant source of the UCM. First, in surface sediments where the unresolved complex mixture of hydrocarbons predominates and sufficient hydrocarbons were isolated to obtain a measure of C-14 activity, the maximum amount of modern carbon present was 20% (163, 164, 169, 170). Second, the isomeric mixture of hopanes in hydrocarbons isolated from surface sediments where the UCM predominated, suggested a non-biosynthetic origin for these triterpane cycloalkanes (171). Third, the biosynthesis of the phenanthrenes, especially in the observed distribution, is unlikely (141-143).

The predominant olefinic compounds at retention index 1700 most likely originated from phytoplankton or benthic algae (33, 34). As far as we know, the unknown alkenes and cycloalkenes at retention index 2000 to 2200 are not present in the predominant plants of the area (2). They probably represent diagenetic alteration products of some organic precursor which may be identified once the structures of the cycloalkenes are elucidated. The 23-31 carbon n-alkanes have the odd carbon predominance characteristic of land plants and marsh plants (2) and are probably from these sources.

(ii) Early Diagenesis of Organic Matter: Surface sediments and early Pleistocene sediments from many continental margin areas have been analyzed for total hydrocarbon content. The general conclusions are that substantial quantities of a complex mixture of alkanes-cycloalkanes and aromatic hydrocarbons are not formed in surface sediments but require increased temperature at depth (167, 172). Our data from the deepest core sections agree with that conclusion. However, it seems that low concentrations can result from natural processes and these must be taken into account in studies of the diagenesis of organic matter in sediments.

The previously discussed C-14 activity determinations and hopane isomer determinations also apply in suggesting that the UCM is not from early diagenesis of recently deposited organic matter. In addition, the distribution of phenanthrenes is not what is expected from early diagenesis (141).

(iii) Ancient Sediments: There are no known major outcrops of ancient sediments contributing material to the sediments we have sampled (173). The distribution of phenanthrenes and other aromatic hydrocarbons is not consistent with an ancient sediment source (141).

(iv) Natural Pyrolytic Sources - Forest Fires: This source is consistent with the distribution of phenanthrenes (141) and the complex mixture of alkanes and cycloalkanes. While we have no historical records of forest fire frequency and severity in New England over time, there are studies of changes in forest expanse, biomass and net primary productivity for 1620 to the present (174). These indicate that trends in decreasing forest acreage, stature, and productivity initiated with European settlement were reversed about 1900. However, subsequent increases have only been on the order of 25 to 30 of the 1900 value (Wood and Botkin, 1976, in preparation). This is less than the factor of 3 to 4 increase noted for phenanthrenes in surface sediments in comparison to sediments deposited circa 1900.

(v) Chronic Oil Spills: If chronic oil spills or oil discharges into the area with the predominant source of the UCM, then the aromatic hydrocarbons would have a distribution similar to that found in petroleum; they do not (141). The possibility of alteration of the relative abundance of homologs within the aromatic hydrocarbons in oil due to weathering and biodegradation should be considered. Studies of spilled oil incorporated into sediments over 1.5 years have shown that the original relative composition of aromatics in oil is not transformed to the distribution observed in the sediments at Station P. Rather, the opposite occurs, as expected from solubility considerations, and the parent plus C_1 to C_3 homologs are lost relative to higher homologs (65, 93, 94). Thus, chronic oil inputs are unlikely predominant sources of the complex mixture of alkanes and cycloalkanes and aromatic hydrocarbons in Station P sediments unless longer term processes produce a dramatic change in the aromatic hydrocarbon distribution within homologous series. We are currently pursuing a longer term study to investigate further this possibility.

(vi) Anthropogenic Pyrolytic Source: The UCM of alkanes and cycloalkanes is present in urban air (175). The distribution of phenanthrenes and other aromatic hydrocarbons present in surface sediments is different from that found in urban air (141-143). In particular, the parent unsubstituted compounds, e.g. phenanthrene, are present in higher abundance relative to substituted aromatics, e.g. methyl and dimethylphenanthrene, in the urban air. This led Youngblood and Blumer (141) to propose that natural pyrolytic sources such as forest fires were a more likely source of the aromatics in surface sediments in areas without oil pollution. Hase and Hites (143) suggest that the argument by Youngblood and Blumer (141) does not take into account the possibility of alteration of urban air fallout after deposition - in particular the decreasing solubilization of the aromatics within a homologous series as the number of alkyl substituent carbons increase. They propose that the aromatic hydrocarbon distribution in surface sediment could result from water washing away proportionately greater amounts of the more soluble parent compounds, thereby converting the urban air particulate aromatic distribution to that observed in the sediments.

If urban air were a predominant source for the complex mixture of alkanes, cycloalkanes and phenanthrenes this would explain the observed increase in concentrations of both groups of hydrocarbons in the upper core sections. The Pb-210 geochronology record shows that the UCM hydrocarbon concentrations increase dramatically at depth in the cores representing the time when fossil fuel combustion increased dramatically in the twentieth century. Buzzards Bay is part of the coastal area adjacent to the highly industrialized northeastern U.S. Indeed, recent ozone measurements suggest that urban air from the New York metropolitan area is transported to the Buzzards Bay area (176).

5. General and Concluding Comments

Our assessment of the available data leads us to suggest that urban air is the predominant source of the complex mixture of alkanes-cycloalkanes and aromatic hydrocarbons in the upper 24 cm of sediment at Station P because this source provides the simplest explanation of the depth distributions. The concentrations in the surface sediment sections reflect increased delivery of combustion products as a result of increased combustion of fossil fuels. However, simplicity is not proof. Forest fires are next in order of most likely source.

Investigations of the depth distribution of DDT (177), lead (178), and metals (179, 180) in varved or Pb-210 dated sediments have led to the conclusion that a historical record of man's inputs of these pollutants to the sediments has been preserved. If the depth distribution of hydrocarbons in the cores we have analyzed represents a historical record of pollutant hydrocarbons over the past several decades, then the benthic ecosystem of the area has been exposed to an increasing level of pollutants. Since these are higher concentrations of DDE and PCBs in the surface sediments (Table 18) in addition to the air pollutant hydrocarbons, the question of synergistic interactions between the two must be raised. Whether or not these pollutants are present in a form that is available for uptake by the benthic organisms is unknown. Further investigation is certainly in order.

Finally, we raise the issue of the presence of low, but detectable, concentrations of a complex mixture of alkanes-cycloalkanes and the aromatic hydrocarbon phenanthrenes in the deep sections of the core which were deposited circa 1780 (based on back extrapolation of the Pb-210 geochronolgy sedimentation rate). Urban air and oil pollutant hydrocarbons are not the sources for these hydrocarbons unless a dramatically different benthos existed in this area at some intermediate time between 1780 and about 1860 (the lowest level of reliable Pb-210 recording of bioturbation) which mixed 1860 to 1900 urban air fallout to a greater depth than the present 3 to 4 cm bioturbated zone (150, 181). It us unlikely they are from inputs since 1955 because Cs-137 and Pu-259 are not detected below 20 cm at this location (182). The complex mixture of alkanes-cycloalkanes and the phenanthrenes in the deepest core section at Station P are probably from natural sources - most likely a pyrolytic source such as forest fires. The presence of low concentrations of these compounds at a time before the industrial revolution in North America means that benthic ecosystems in this area have been exposed to low concentrations of the polynuclear aromatic phenanthrenes prior to man's input and that the input from man's chronic release of these compounds to the environment has resulted in an increase of an order of magnitude.

SECTION 10

LIMITED STUDY OF A NO. 2 FUEL OIL SPILL

1. Introduction

The West Falmouth oil spill study (63-66, 93, 94) was among the first to apply sensitive and discriminating analytical chemistry techniques and biogeochemical interpretation to oil pollution investigations. There have been several discussions of the results of this spill at scientific meetings and in the press which suggested that this oil spill was unique in that the petroleum compounds survived for such a long period of up to two and one-half years after the spill. Thus, when another No. 2 fuel oil spill occurred in Buzzards Bay, Massachusetts at about the same time of the year - September, 1969 vs. October, 1974 - and oil came ashore in an area not far removed from the areas of the West Falmouth oil spill (Figure 30) we took advantage of this "duplicate" experiment.

Our intent was to sample at two intertidal stations and compare the fate of this oil with the fate of the oil spilled into intertidal areas by the West Falmouth oil spill. We did not intend a comprehensive spatial or temporal sampling program because of limitations on funding and laboratory space.

2. Methods

Marsh and intertidal mudflat sediments were obtained by coring with a 1 meter x 6 cm diameter stainless steel coring device which was driven into the sediments with a 34 kg sledge hammer. Surface sediments were obtained by clean stainless steel spatula or spoon. All samples were obtained within one hour of low tide. Cores were sectioned in the laboratory and frozen at -4°C until analysis.

Extraction, column chromatography and gas chromatographic analysis procedures have already been described in secton 5 or section 6. In addition, we recently reanalyzed several samples using glass capillary gas chromatography and glass capillary mass spectrometry computer systems. Fraction 2 aromatic compounds were analyzed on a 0.32 mm i.d. x 20 m glass capillary SE 52 column installed in a Hewlett Packard Model 5710 A gas chromatograph equipped with a flame ionization detector and Model 18740 A capillary column split/splitless injector. Samples were injected using the splitless mode with column at 40°C and held at that temperature until the solvent peak eluted. Then the column was rapidly heated to 70°C and then immediately programmed at 2°/min to 240°C. Gas flow rate was 2-3 ml/minute helium. Detector temperature was 250°C and injection port temperature was 200°C.

Quantitation was achieved by comparison of peak heights of compounds in samples with peak heights of an internal standard, hexamethyl benzene or by comparison with an external standard calibration plot of several aromatic hydrocarbon standards.

The aromatic compounds were also analyzed by GC-mass spectrometry on a Finnigan 1015 quadrupole mass spectrometer coupled to a Varian 1400 gas chromatograph equipped with a split/splitless injection. A 17 m x 0.32 mm i.d. SE 52 glass capillary column was installed in the system and operated as follows. Injection was at 40°C with 30 sec. in splitless mode, then rapidly brought to 70°C and programmed to 240°C at 4°/min. Helium carrier gas was at 14 psi. The mass spectrometry operating conditions were 20 eV, 500 ua, scan from 40-500 amu every three seconds. Mass fragmentographic quantitative analyses were obtained using the general procedure outlined in section 8 except that the precision was only + 20 due to the small number of scans per peak due as a result of long scan time. Mass spectra for each peak selected for analysis were compared where possible with the API reference spectra and compounds identified in this manner. In many cases reference spectra were not available and compounds were tentatively identified by comparison of their spectra with known spectra for isomers or compounds with similar structure characteristics.

3. Results and Discussion

The concentrations of f_1 and f_2 compounds in the marsh and intertidal sediments are presented in Tables 21 and 22. Note that concentrations of hydrocarbons decrease markedly between October, 1974, the month of the spill, and May, 1975. Also note that concentrations of hydrocarbons are much greater in the surface sediments than those deeper in the cores. As time progresses the concentrations of hydrocarbons decrease.

Duplicate analyses are given in Tables 21 and 22 for several core sections to indicate the combined geochemical and analytical variability of the data. Most of this variability is contributed by sample heterogeneity inherent in marsh sediments and intertidal sediments. We also think that uneven distribution of the fuel oil in the sediments contributes to this variability.

Curiously, the concentrations at Site G.H. increased by a factor of 10 from May, 1975 to July, 1977. We think this reflects mobilization of oil from another area into this area since it is adjacent to a small stream. However, we cannot be certain as the spatial sampling did not allow for such an investigation as previously mentioned.

Gas chromatograms representative of the samples are presented in Figures 37-41. Note that there is a very good match of the No. 2 fuel oil gas chromatogram with the gas chromatograms of the October, 1974 marsh sediment sample (Figure 37) and with the intertidal sand sample (Fig. 38). However, by May of 1975 the gas chromatograms of the surface sediment sample for the intertidal sand (Figure 39) shows some depletion of the

TABLE 21. CONCENTRATIONS OF HYDROCARBONS IN MARSH SEDIMENTS: NO. 2 FUEL OIL SPILL, WINSOR COVE (mg/g DRY WEIGHT).

LOCATION		DATE	f_1 (ALKANES + CYCLOALKANES)	f_2 (AROMATICS)	TOTAL
Core Site 1		10/74			
0-6 cm	a		61.1	20.6	81.7
	b		32.8	11.4	44.2
14-18 cm	a		.32	.12	.44
	b		.79	.24	1.03
		5/75			
0-5 cm			14.3	4.6	18.9
15-20			.18	.05	.23
		5/76			
0-6 cm			11.8	5.1	16.9
25-29 cm			.03	.06	.09
45-49 cm			.06	.06	.12
		6/77			
0-7 cm			2.29	.32	2.61
Site G.H.					
0-7 cm		5/75	.86	.37	1.23
0-7 cm	a	7/77	3.48	3.04	6.52
	b		10.00	1.31	11.31
Control - Chappaquoit					
0-7 cm		5/75	.04	.03	.07
0-7 cm		7/77	.02	.04	.06

TABLE 22. CONCENTRATION OF HYDROCARBONS IN SANDY SEDIMENTS: NO. 2 FUEL OIL SPILL, WINSOR COVE (μg/g DRY WEIGHT).

SAMPLE DATE		f_1 (ALKANES + CYCLOALKANES)	f_2 (AROMATICS)	TOTAL
October, 1974				
0-2 cm	a	58.3	17.2	75.5
	b	41.3	19.2	60.5
20-24 cm		30.0	6.0	36.0
48-52 cm		20.7	2.9	23.6
May, 1975				
0-4 cm		168.0	46.7	214.7
22-27 cm		10.6	3.4	14.0
37-45 cm		20.1	5.8	25.9
October, 1976				
Surface 0-2 cm	a	18.4	39.7	58.1
	b	92.8	31.7	124.5
	c	60.1	22.4	82.5
	d	203.0	194.5	397.5

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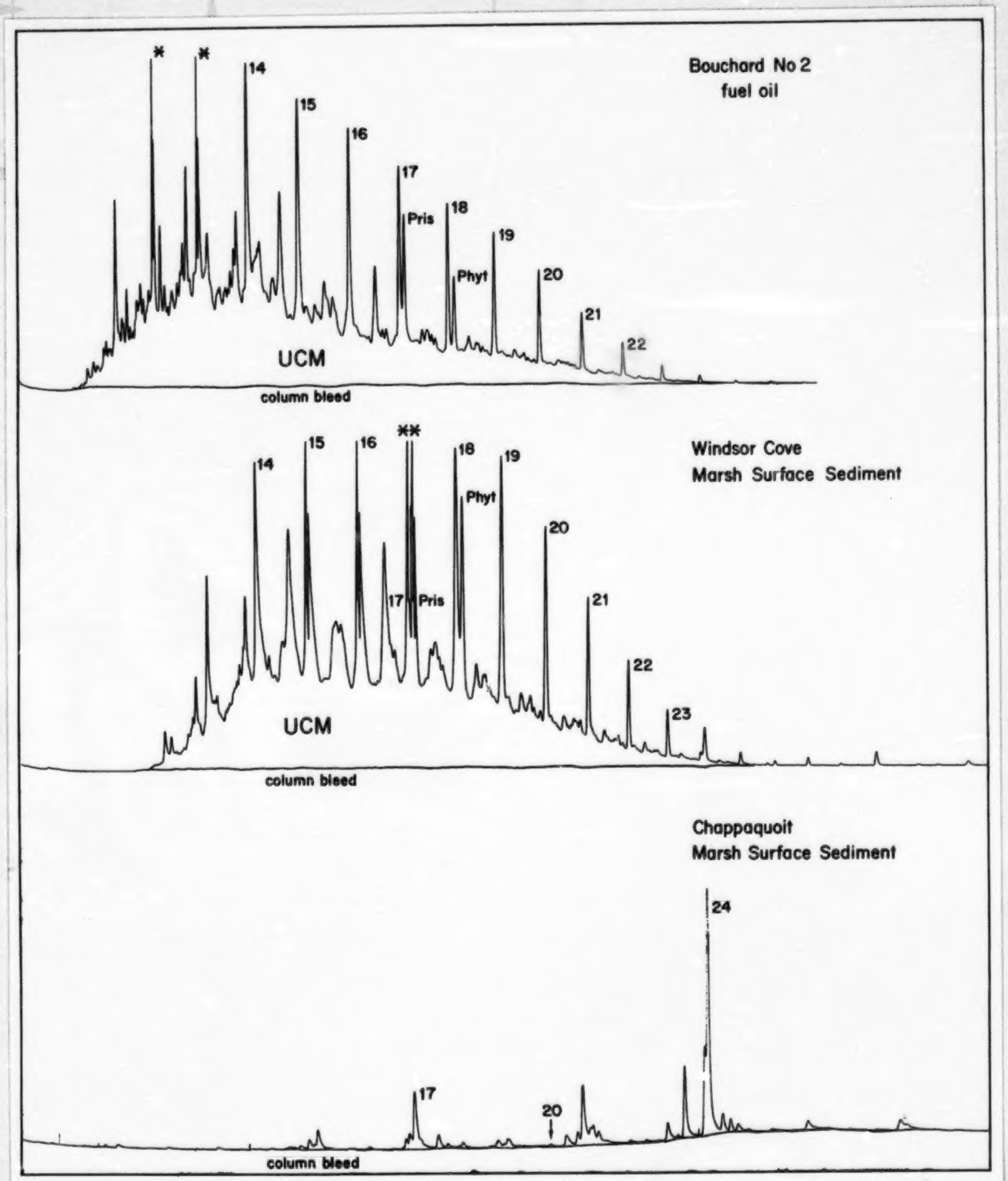

Figure 37. Gas chromatograms of alkanes and cycloalkanes of Bouchard No. 2 fuel oil, Windsor Cove surface sediment, and Chappaquoit control station surface sediment.

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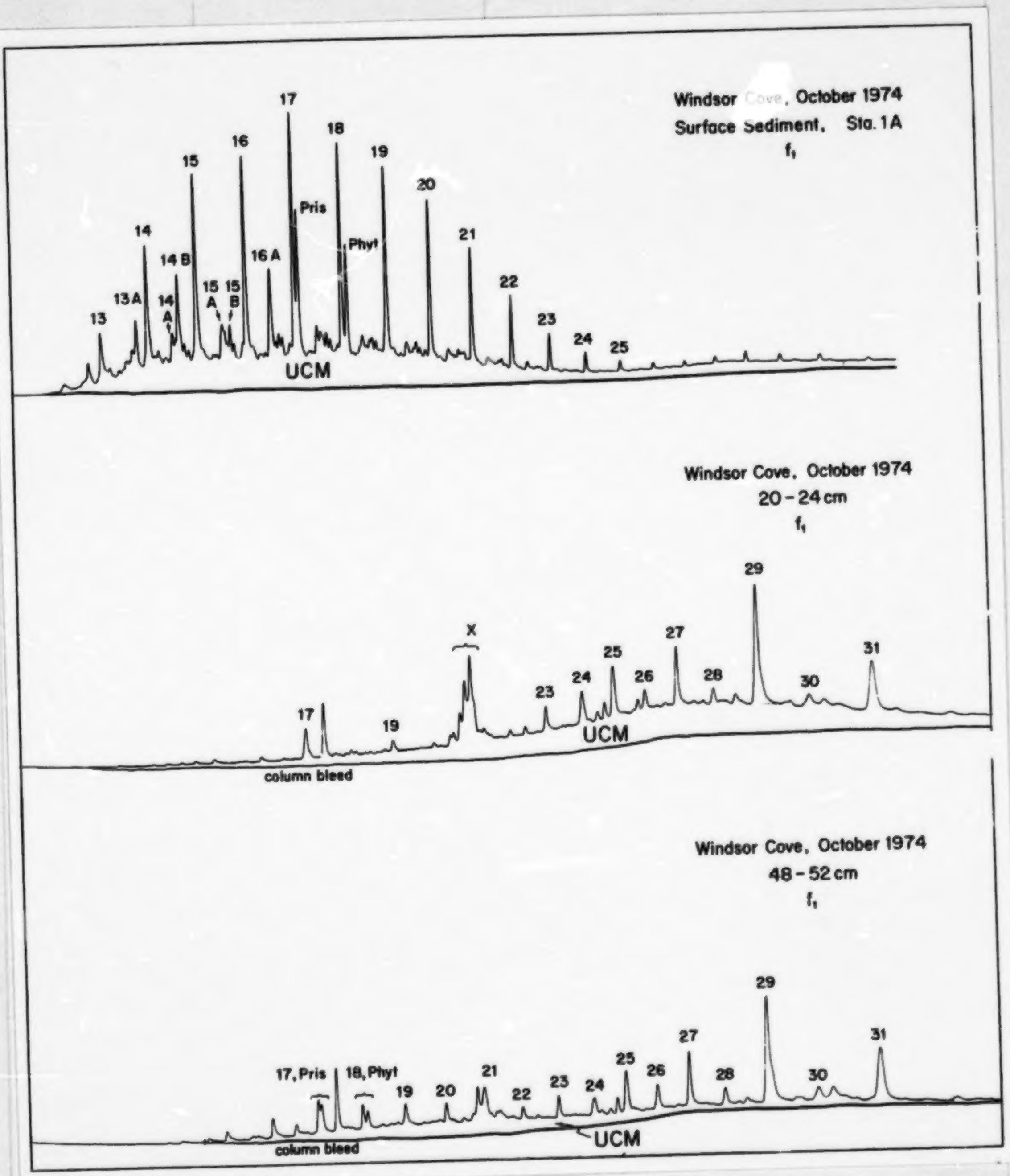

Figure 38. Gas chromatograms of alkanes and cycloalkanes of October, 1974 intertidal sand core, Windsor's Cove oil spill.

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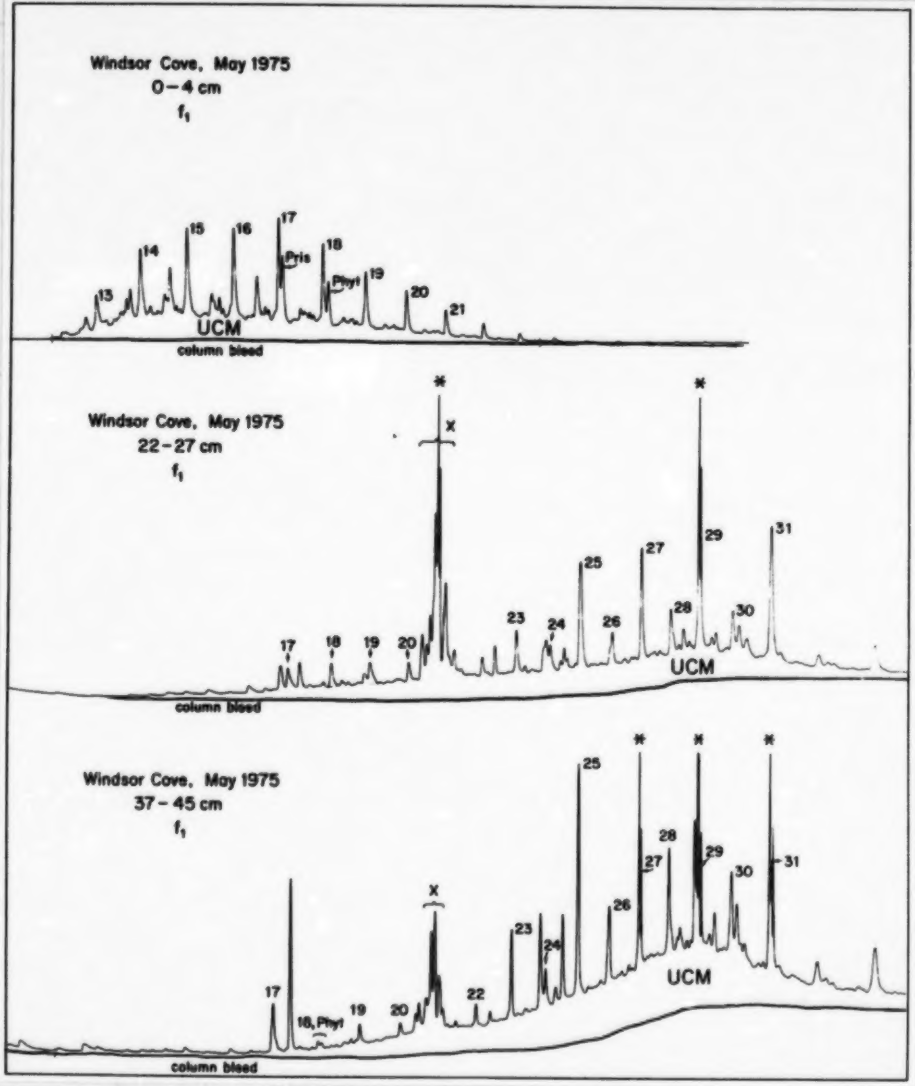

Figure 39. Gas chromatograms of alkanes and cycloalkanes of May 1975 intertidal sand core Windsor's Cove oil spill.

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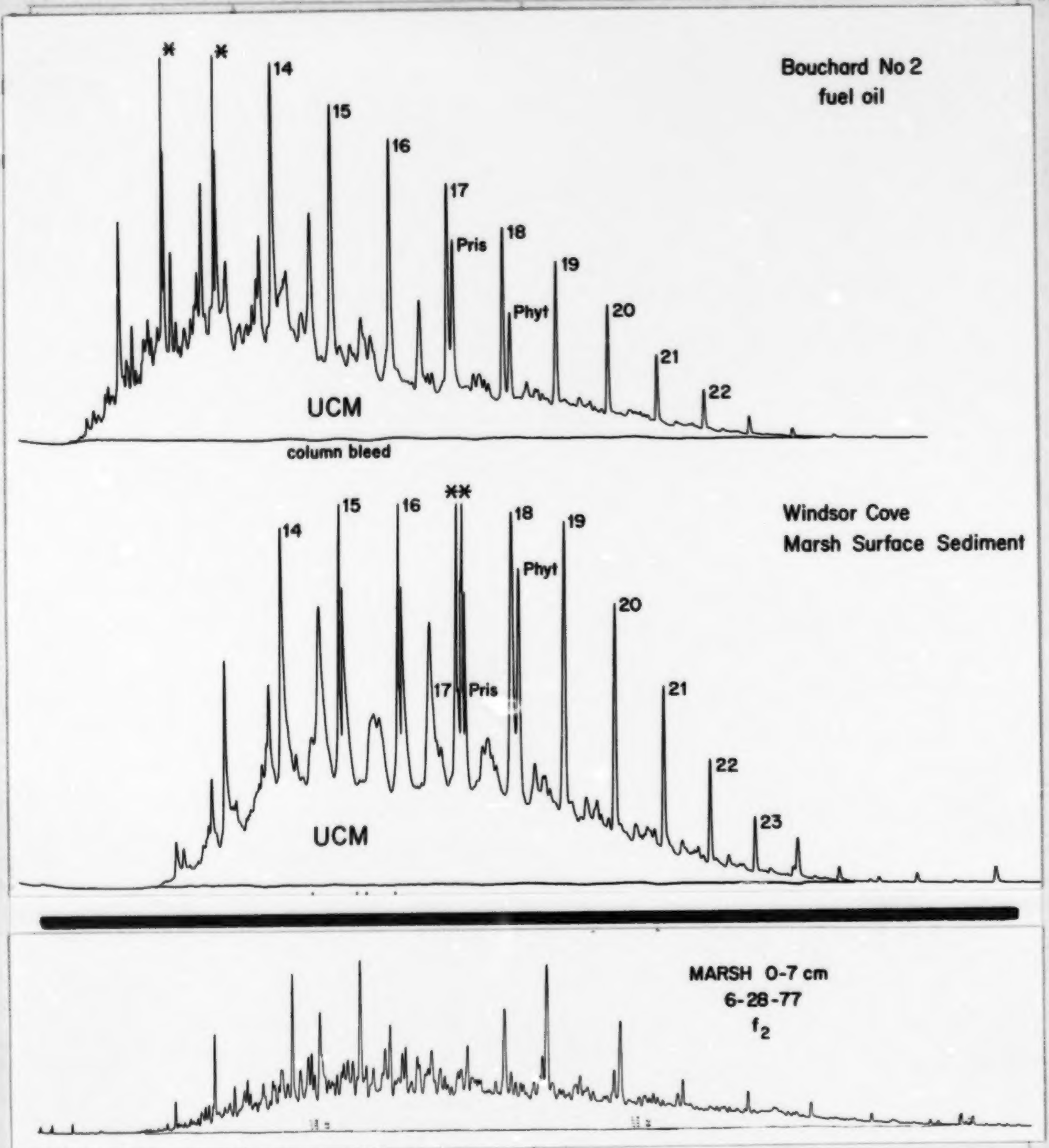

Figure 40. Gas chromatograms of alkanes and cycloalkanes of Bouchard No. 2 fuel oil, marsh surface sediment October 1974 and marsh surface sediment June 1977, Windsor's Cove oil spill. The 1977 chromatogram was run using an SE-52 glass capillary column.

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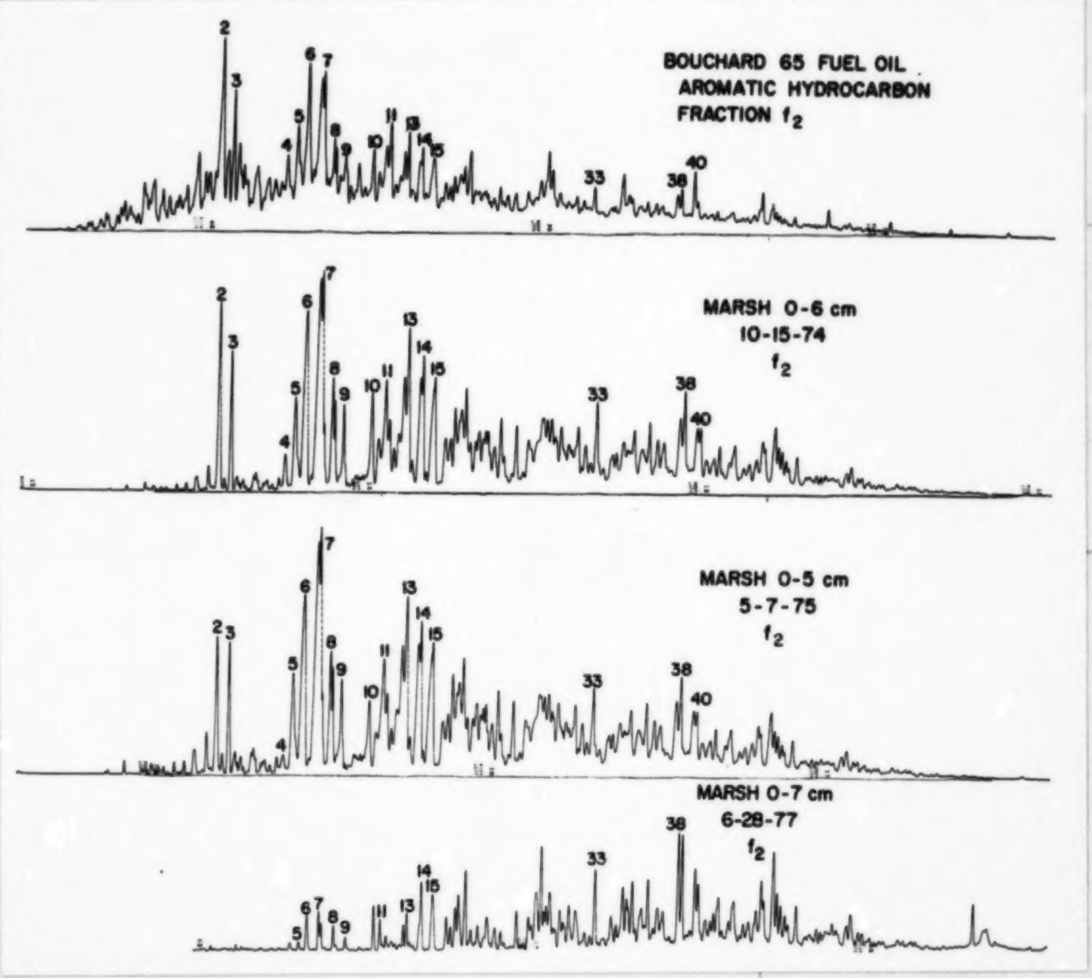

Figure 41. Gas chromatograms of aromatic hydrocarbons of Bouchard No. 2 fuel oil and marsh surface sediments October 1974, May 1975, and June 1977. See Table 24 for peak identifications. (Glass capillary SE-52 column).

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n-alkanes relative to the isoprenoid hydrocarbons pristane and phytane. Comparison of Figure 37 with Figure 40 shows that by June, 1977 the n-alkanes in the marsh sediment are severely depleted relative to the isoprenoid alkanes. This is the pattern expected by microbial attack on the oil (1, 2, 35, 57, 64-66, 97, 108) and is the exact same pattern of events as observed at the West Falmouth oil spill site.

The gas chromatograms of f_1 hydrocarbons in the deeper core sections show the background pattern of hydrocarbons in the intertidal sand and the gas chromatogram in Figures 37 and 38 shows the gas chromatogram expected from an open, non-grassy area of a marsh, i.e. mainly benthic diatom, and bacteria hydrocarbons (Figure 37), a grassy area with the C_{23} to C_{31} n-alkanes predominating as expected (see section 4). Comparison of gravimetric and gas chromatography data for several of the oil spill samples is given in Table 23. Note that there is good agreement as should be the case when the bulk of the hydrocarbons are in the molecular weight range of the gas chromatographic analyses as is the case when No. 2 fuel oil compounds predominate in the hydrocarbon fractions.

Glass capillary gas chromatograms of the aromatic compounds in fraction 2 are presented in Figures 42 and 43. Note the following - first, the relative abundance of the naphthalene, methyl naphthalenes, and dimethyl naphthalenes decrease dramatically relative to the methyl phenanthrenes (see Table 24 for compound identifications). Second, there are still detectable concentrations of No. 2 fuel oil aromatic hydrocarbons in the marsh sediment after three years of weathering and microbial attack. Fourth, a comparison of the May, 1975 marsh surface sediments and the May, 1975 intertidal sand surface sediments shows that the sand sediments (Figure 43) had much more depletion of the methyl and dimethyl naphthalenes. This is expected as it would be easier to wash the oil components out of coarser grain lower organic matter content sands than from the organic rich marsh sediments of decaying roots and fine grained minerals.

The concentrations of selected groups of aromatic hydrocarbons in the marsh sediment are given in Table 25. This data shows the rapid initial decrease in total concentration of aromatic hydrocarbons followed by a period of slower decrease in concentration. During this later phase, there is a faster decrease of lower molecular weight compounds such as the methyl and C_2-naphthalenes relative to the C_3-naphthalenes and methyl phenanthrenes.

Gas chromatography-mass spectral data representative of that obtained by glass capillary gas chromatography-mass spectrometry are given in Figures 43-44. This is indicative of the most modern (1977) analyses available. Comparison of the gas chromatograms of Figures 43-44 with Figure 45 clearly demonstrate the advances in resolution and information gathering capability which have evolved since the formal end of this grant period in 1976 and our work in the summer of 1977 with funding from other grants.

TABLE 23. COMPARISON OF GRAVIMETRIC AND GAS CHROMATOGRAPHIC ANALYSES FOR OIL SPILL CONTAMINATED SEDIMENTS FROM WINSOR COVE (μg/g DRY WEIGHT).

		GRAVIMETRY	GAS CHROMATOGRAPHY
October, 1974 marsh 0-6 cm f_1 duplicates	a	32,800	21.650
	b	61,100	76,680
May, 1975 marsh 0-5 cm f_1		14,300	14,500
May, 1976 marsh 0-9 cm f_1		11,800	24,400
f_2		5,100	8,900
October, 1974 sand 0-2 cm f_1 duplicates	a	58.3	31.1
	b	41.3	26.6
May, 1975 sand 0-4 cm f_1		168	184

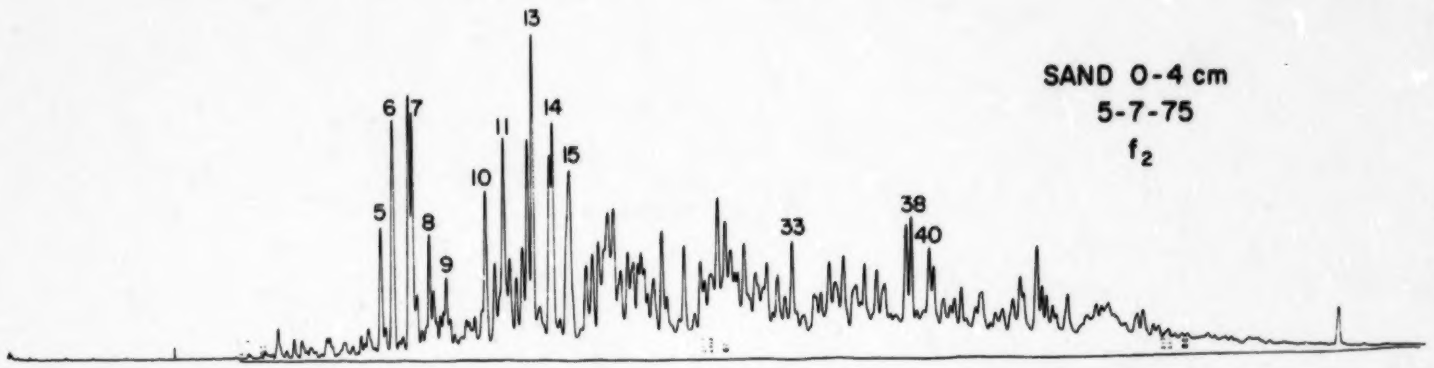

Figure 42. Gas chromatogram of aromatic hydrocarbons in Windsor Cove intertidal surface sand. (Glass capillary SE-52 column)

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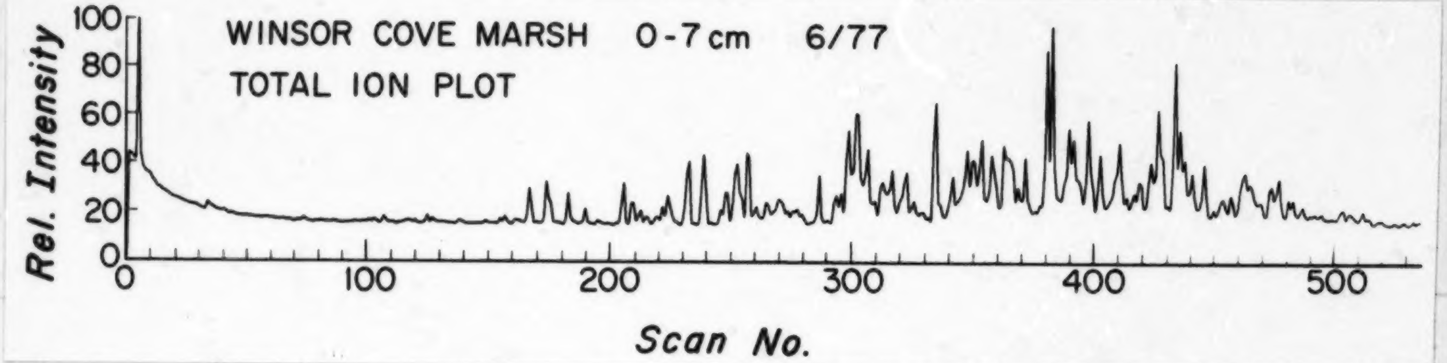

Figure 43. Gas chromatography-mass spectrometry total ion plot, aromatic compounds surface sediment, June 1977, Windsor's Cove. Glass capillary column.

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TABLE 24. IDENTIFICATION OF PEAKS IN FIGURES 41 AND 42.

PEAK NO.	COMPOUND	IDENTIFICATION BY:
2	2-methylnaphthalene	GC and MSA
3	1-methylnaphthalene	GC and MSA
4-9	C_2-naphthalenes	MSA
10-15	C_3-naphthalenes	MSA
33	phenanthrene	GC and MSA
38	methylphenanthrene	MSA
40	methylphenanthrene	MS

GC - gas chromatography retention indices.

MS - mass spectral interpretation.

MSA - mass spectra match with API reference spectra.

TABLE 25. CONCENTRATIONS OF NAPHTHALENES AND PHENANTHRENES IN WINSOR COVE MARSH SURFACE SEDIMENTS 0-6 cm (μg/g DRY WEIGHT).

COMPOUNDS	OCTOBER, 1974	MAY, 1975	JUNE, 1977
Naphthalene	9.2	0.63	0.11
C_1-naphthalenes	370	41	1.2
C_2-naphthalenes	1380	260	15
C_3-naphthalenes	3040	590	33
C_1-phenanthrenes	500	97	15
C_2-phenanthrenes	480	96	27
Amounts of Naphthalenes and Phenanthrenes in Surface Sediments from Winsor Cove, Normnalized to C_3 Naphthalenes.			
C_1-naphthalenes	.12	.07	.04
C_2-naphthalenes	.45	.44	.45
C_3-naphthalenes	1.00	1.00	1.00
C_1-phenanthrenes	.16	.16	.46
C_2-phenanthrenes	.16	.16	.82

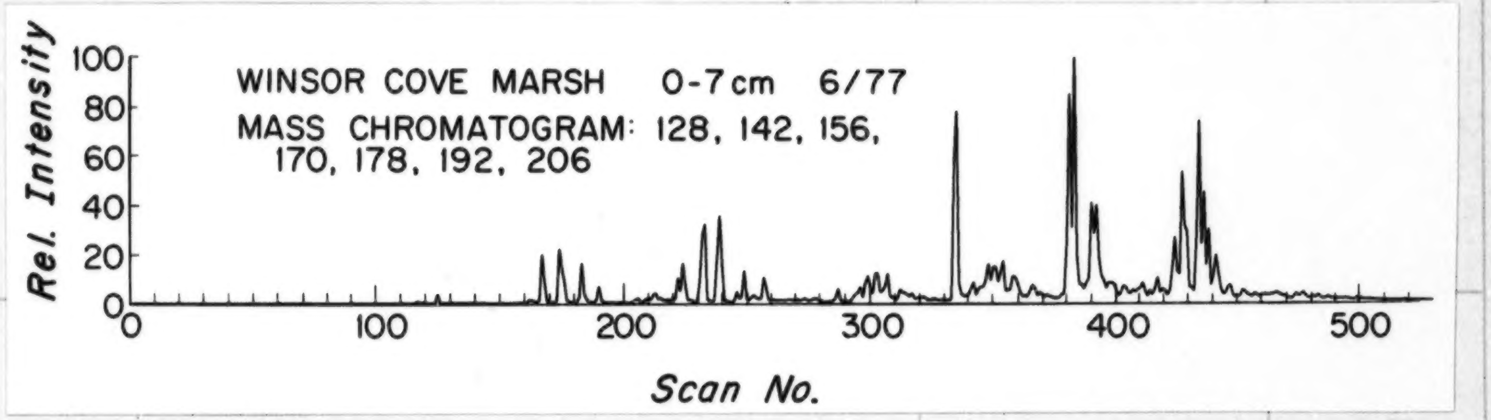

Figure 44. Sample of Figure 43-selected ion plot for m/e 128, 142, 156, 170, 192, and 206.

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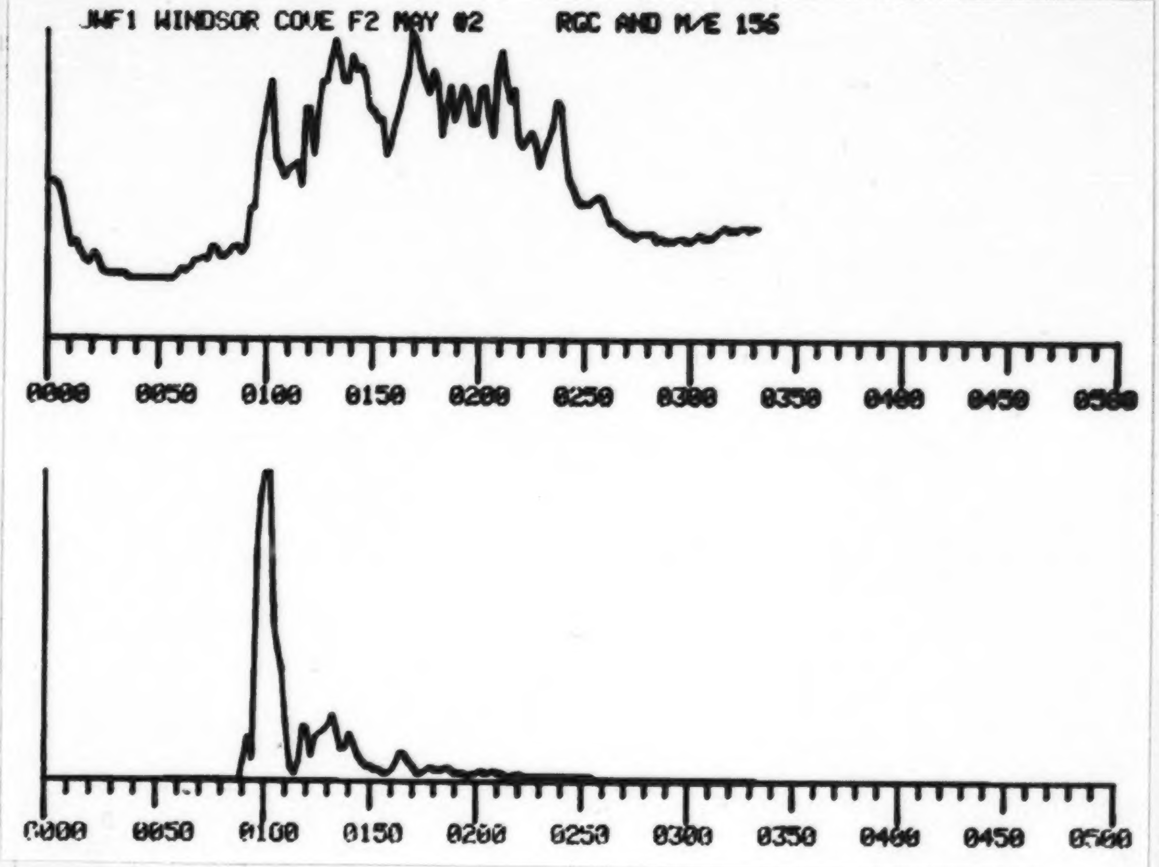

Figure 45. Gas chromatography-mass spectrometry total ion and m/e 156 plot of aromatic fraction of marsh surface sediment, May 1976, Windsor's Cove. Packed glass column.

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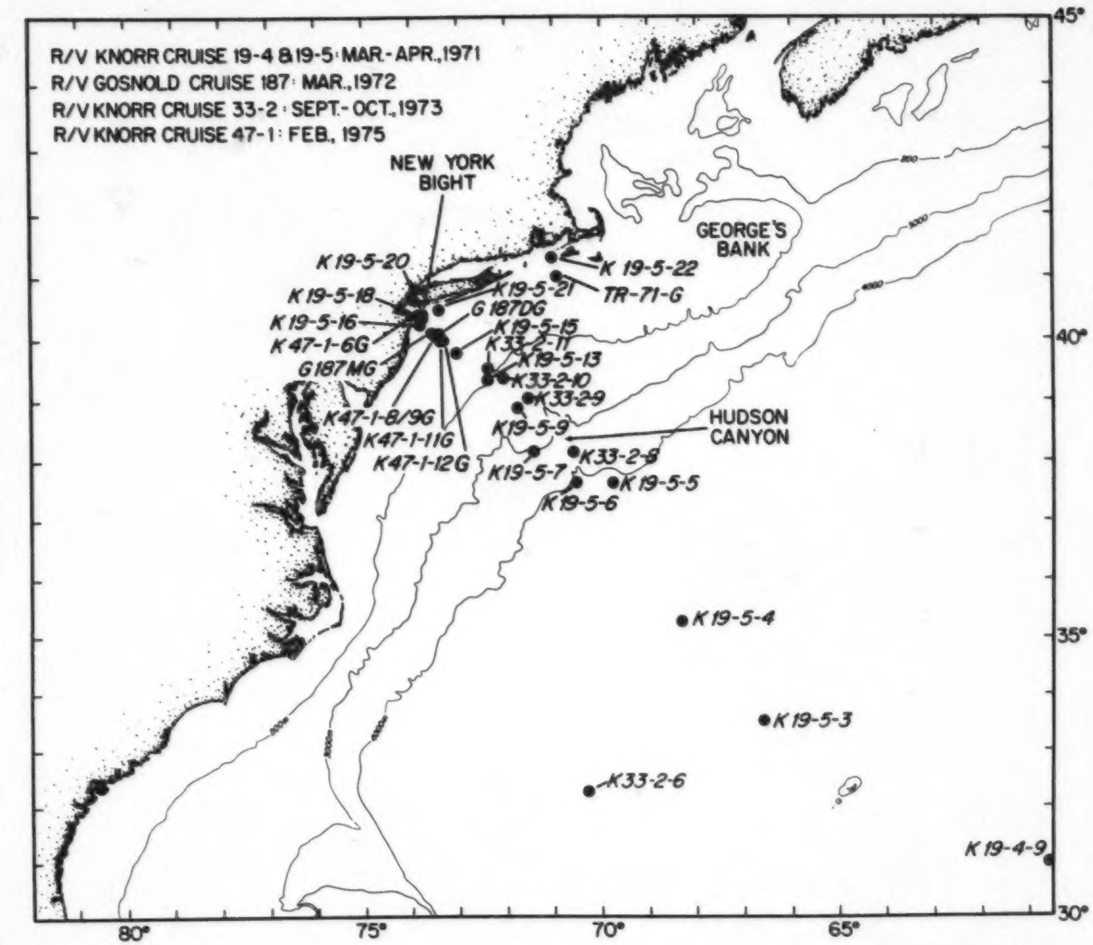
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R/V GOSNOLD CRUISE 187: MAR.,1972
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R/V KNORR CRUISE 47-1: FEB., 1975
NEW YORK BIGHT
GEORGE'S BANK
HUDSON CANYON
K 19-5-20
K 19-5-18
K 19-5-16
K 47-1-6G
G 187 MG
K47-1-8/9G
K47-1-11G
K47-1-12G
K 19-5-22
TR-71-G
K19-5-21
G 187DG
K19-5-15
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Figure 46. Sampling locations for analyses of hydrocarbons in surface sediments in the Western North Atlantic.

It is important to make this comparison because many of the instrumental analysis techniques we employed in the initial stages of the grant have been rendered less desirable for oil pollution studies. In particular, the use of glass capillary chromatography interfaced with mass spectrometer computer systems has tremendous advantages over the SCOT column gas chromatography and packed gas chromatography-mass spectrometry we originally used.

We also conducted some spiking experiments with Winsor Cove sediments. Intertidal sand and marsh core sediments which had been previously extracted were spiked with No. 2 fuel oil from the Bouchard Barge No. 65 which spilled this oil into the Winsor Cove area. The samples were analyzed as described in section 6.

The results are given in Table 26. The recoveries of the No. 2 fuel oil ranged from 50 to 69%. This was better than the recoveries of 30% reported for API No. 2 fuel oil spiked to surface sediments from Station C, Narragansett Bay, Rhode Island (see Table 12, section 6). This is probably due to the higher concentration of these spikes - 300 to 800 μg/g dry weight - compared to the 10 μg/g dry weight of the API No. 2 fuel oil spikes. The suspected reasons for this recovery range 30 to 70% are given in section 6.

We have briefly examined the usefulness of the gas chromatographic passive tagging parameters, the ratios of n-C_{17}/pristane, n-C_{18}/phytane, pristane/phytane, and n-C_{17}/n-C_{18}. The ratios for the surface sediment samples from Winsor Cove and the Winsor Cove fuel oil spiked samples are given in Table 27. The results are not very encouraging for the use of these parameters for passive tagging. Even at fuel oil concentrations of 300 to 30,000 μg/g dry weight of sediment, in sediments sampled three days after an oil spill the passive tagging parameters are changed. These changes were not simulated to any appreciable

TABLE 26. HYDROCARBON CONCENTRATIONS FOR WINSOR COVE SEDIMENTS SPIKED WITH BOUCHARD NO. 2 FUEL OIL.

	SAND 37-45 cm CORE SECTION	SAND 22-27 cm CORE SECTION	MARSH 14-18 cm CORE SECTION
Concentration No. 2 fuel oil spike added μg/g dry weight	334	375	850
Analyses (μg/g dry wt.)			
f_1 gravimetry	143	223	345
f_2 gravimetry	26.9	35.2	76.5
Total f_1 and f_2 % of spike recovered	50.9 %	68.9 %	49.6 %

TABLE 27. PASSIVE TAGGING GAS CHROMATOGRAPHY RATIOS FOR NO. 2 FUEL OIL IN WINSOR COVE SEDIMENTS.

SAMPLE	RATIOS $n\text{-}C_{17}$/PRISTANE	 $n\text{-}C_{18}$/PHYTANE	 PRISTANE/PHYTANE	 $n\text{-}C_{17}/n\text{-}C_{18}$
Bouchard No. 2 fuel oil	1.28	1.60	1.92	1.21
Winsor Cove spiked sediments				
sand 37-45 cm	.78	1.31	1.25	.74
sand 22-27 cm	.77	1.18	1.45	.95
marsh 14-18 cm	.81	1.14	1.36	.96
Oil spill samples				
October, 1974 marsh surface sediments	.75	1.83	1.38	1.39
October, 1974 sand surface sediments a	1.26	1.64	1.52	1.16
b	1.56	2.03	1.51	1.16
May, 1975 marsh surface sediments	1.42	1.62	1.39	1.23
May, 1975 sand surface sediments	1.32	1.57	1.48	1.25

degree by spiking sediments with authentic fuel oil and then subjecting them to the same analysis procedures. This is probably a result of many interacting geochemical and biochemical processes acting on the fuel oil compounds, e.g. evaporation, dissolution, selected biodegradation.

It may be that other passive tagging parameters would not be affected too quickly, that is within three days. We doubt this and think that exact match passive tagging of oil incorporated into surface sediments is not a probable occurrence - at least for the types of samples we have investigated.

4. General Conclusions

We have verified the results of the studies of the fate of No. 2 fuel oil in sediments obtained by the West Falmouth oil spill study (64-66, 93, 94) and extended them to include a more discriminating look at the aromatic hydrocarbons. We have recently cooperated with our colleagues Dr. John Teal and Dr. Kathryn Burns in extending their aromatic hydrocarbon analyses of the West Falmouth oil spill marsh samples to include our latest analytical glass capillary-mass spectrometry. The results of our combined efforts have been recently submitted for publication (183).

SECTION 11

HYDROCARBONS IN SURFACE SEDIMENTS OF THE WESTERN NORTH ATLANTIC

We have analyzed a number of surface sediments in the western North Atlantic (Figure 46) for hydrocarbon concentrations and distributions. This effort was primarily supported by the Office of Naval Research, the National Science Foundation and the Office for the International Decade of Ocean Exploration of the National Science Foundation. As part of this effort we sampled sediments in the New York Bight dump site areas and analyzed them for hydrocarbons. This was partially supported by this grant from the Environmental Protection Agency.

The results of the total study have been submitted for publication (169) and is in press. The abstract is presented here.

Abstract. High concentrations of hydrocarbons (500-3000 μg/g dry weight) together with a complex and wide molecular weight range of composition, and low ^{14}C activity of $\delta^{14}C = -833 + 50\ ^{\circ}/_{\circ\circ}$ strongly indicate that fossil fuel hydrocarbons make up the bulk of hydrocarbons in and near the New York Bight dump site areas. The composition and concentrations of hydrocarbons in Hudson Channel sediments suggest some transport of dump site hydrocarbons to areas in the channel midway to the edge of the continental shelf. Sediments at 16 stations in other areas of the continental shelf, continental slope and abyssal plain have hydrocarbon concentrations and compositions indicating that no more than 1 μg/g dry weight of fossil fuel hydrocarbons are present.

N-alkanes from land sources were among the most predominant hydrocarbons in all samples except those in the New York Bight region. Their presence in abyssal plain surface sediments documents transport of some land derived organic matter to these deep ocean areas. Two 25 carbon cycloalkenes are among the more predominant hydrocarbons in continental shelf surface sediments except for the dump site areas. These two compounds are present in lower concentrations in slope sediments and are not detected in abyssal plain sediments.

SECTION 12

U.S. AND U.S.S.R. JOINT PROGRAM FOR THE INTERCALIBRATION OF METHODS OF ANALYSIS FOR OIL AND OIL BY-PRODUCTS

The Principal Investigator, Dr. Farrington, and Dr. Peter Rogerson, EPA Project Officer for this grant, travelled to the Soviet Union October 2-17, 1975 to discuss analytical and geochemical problems associated with oil pollution studies. A report of this trip, discussions, and arrangements were presented via normal report channels for procedures and a copy is not included here.

During and after the project period, Dr. Farrington continued to attend meetings and advise EPA on this matter.

SECTION 13

GENERAL DISCUSSION OF PROBLEMS ENCOUNTERED DURING THE PROJECT

1. Gas Chromatographic Analyses Using SCOT Columns

We experienced considerable problems when attempting to analyze small amounts of individual hydrocarbons at the 1 to 10 ng on the SCOT OV-101 and OV-1 columns. Consultation with Perkin-Elmer Corporation gas chromatographic analysis specialists convinced us that we were trying to push the columns beyond their intended analysis range. This was particularly the case for the aromatic hydrocarbon fractions. We devoted considerable effort towards attempting to analyze the aromatic hydrocarbons by SCOT column. However, standards did not routinely chromatograph well and we were forced to abandon this effort. This was unfortunate and did not allow us to have a good data base for comparison of results of analyses for aromatic hydrocarbons with results for the alkanes and cycloalkanes.

Since our efforts, routine glass capillary gas chromatography has become feasible (185) and we think there is no need to further consider SCOT metal column analyses for oil pollution studies.

2. Interferences of Lipid Class Compounds with Analyses of Aromatic Hydrocarbons

We occasionally had problems with interference of methyl ketones (tentative identification) and methyl esters of fatty acids with gas chromatographic analyses of aromatic fractions f_2 and f_3 (see section 5) for definitions. Rechromatography through the alumina and silica column (see section 5) solved this problem.

3. Lack of Experiments with Crude Oils

The problems with metal SCOT column gas chromatography described above, especially for some of the aromatic hydrocarbon fractions limited us to the fuel oil studies reported in the previous sections. We do not view this as a serious drawback because the interpretations and conclusions from the No. 2 fuel oil studies generally apply to fuel oils, crude oils, and Bunker C oils.

We have noted problems in detecting low concentrations of No. 2 fuel oil added to Station C and Station E clams and Station C sediments (sections 5 and 6) because of hydrocarbons already present in sediments. These problems can be expected to be equally as difficult for crude oils and Bunker C oils and in cases of heavier molecular weight compounds even more difficult.

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